Universidade de Aveiro Departamento de Ambiente e Ordenamento
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Sílvia Regina Marques Faria Estrela

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências e Engenharia do Ambiente, realizada sob a orientação científica do Doutor Jan Jacob Keizer, Investigador Auxiliar do CESAM no Departamento de Ambiente e Ordenamento da Universidade de Aveiro. A co-orientação foi realizada pelo Doutor Valdemar Inocêncio Esteves, Professor do Departamento de Química da Universidade de Aveiro, e pelo Doutor José Maria De la Rosa Arranz, Investigador Auxiliar no *Instituto de Recursos Naturales y Agrobiología de Sevilla*.

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A Deus que me fortalece, que me guia todos os dias e que nunca me deixa desistir.

o júri

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Investigador auxiliar, Universidade de Aveiro (**Orientador**)

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WHAT PEOPLE THINK THESIS WRITING IS LIKE:
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WHAT I THINK THESIS WRITING IS LIKE:
WHAT THESIS WRITING IS REALLY LIKE:
tap *yawn* tap tap tap
tap tap tap tap tap
click click

palavras-chave

Carbono, eucalipto, fogos florestais, perda de sedimentos, carbono orgânico dissolvido, carbono orgânico particulado, erosão, técnicas analíticas, biomarcadores

resumo

O solo é considerado o maior reservatório de carbono (C) global e, um importante sumidouro de CO_2 atmosférico. Os incêndios florestais são um fenómeno frequente nos ecossistemas mediterrânicos, em especial em Portugal. Nas últimas décadas verificou-se um aumento do número de incêndios e os cenários de alterações climáticas sugerem que os regimes de incêndios se poderão intensificar no futuro. Os incêndios florestais podem provocar efeitos importantes a curto e médio prazo em fatores chave da qualidade do solo, tais como a quantidade e qualidade da matéria orgânica (SOM). Devido à grande quantidade de carbono (C) armazenado no solo, mesmo pequenas mudanças na SOM poderão ter um efeito significativo sobre os ciclos biogeoquímicos e, consequentemente, sobre o clima global. Embora existam vários estudos que documentam os efeitos pós-fogo sobre os processos hidrológicos e de erosão, em termos de impactos sobre a quantidade (em termos de *stocks* e perdas de carbono orgânico (OC) por escorrência superficial), qualidade da SOM e sedimentos exportados, bem como a sua recuperação pós-fogo tem sido pouco estudados. Estes foram os principais objetivos deste estudo, realizado em plantações de eucalipto *(Eucalyptus globulus*), um dos tipos de vegetação florestal mais suscetíveis ao fogo no centro-norte de Portugal.

O efeito dos incêndios florestais na qualidade da SOM do solo foi avaliado na camada superficial do solo (0-2 cm) em 4 períodos de amostragem, imediatamente antes das primeiras chuvas até dois anos após o incêndio. Para tal, foram utilizadas várias técnicas analíticas, tais como a deteção e caraterização de biomarcadores lipídicos por cromatografia gasosa/espetrometria de massa (GC-MS), caraterização de SOM por pirólise acoplada à cromatografia gasosa e à espetrometria de massa (Py-GC/MS) e, por ressonância magnética nuclear ^{13}C de estado sólido (^{13}C NMR). As exportações pós-fogo de OC por escorrência superficial e as respetivas contribuições das frações de carbono orgânico dissolvido (DOC), carbono orgânico particulado (POC) e carbono inorgânico dissolvido (DIC) foram também determinados em amostras de escorrência superficial recolhidas em intervalos de 1 a 2 semanas ao longo do primeiro ano após o incêndio.

Os resultados mostraram que o incêndio provocou mudanças consideráveis na quantidade e qualidade da SOM. Estas incluíram a degradação térmica e quebra de compostos de *n*-alquilo. Aumentaram os rácios das cadeias curto-longo de *n*-alcanos e das cadeias de *n*-FAMEs, assim como a alteração dos respetivos índices. Além disso, a abundância relativa de certos biomarcadores específicos de determinadas plantas foram modificados, especialmente diminuição de terpenóides, tais como epiglobulol, ledol e globulol que são característicos do *Eucalyptus globulus*. Outras diferenças observadas no solo queimado foram a presença de levoglucosano, um marcador típico para a alteração térmica de polissacarídeos, maior abundância relativa de compostos derivados da lenhina (vanilina e metoxifenol) e a presença de estruturas de N-heteroaromáticos. Os espetros de ^{13}C NMR também indicaram que o fogo produziu um aumento considerável na aromaticidade e condensação aromática da SOM. Estas diferenças verificaram-se durante o período de estudo, sugerindo uma lenta recuperação das propriedades do solo, possivelmente influenciadas, quer por uma recuperação limitada da vegetação, quer pela intensificação das perda de solo após o incêndio.

O presente trabalho abordou também um tema pouco estudado como são os efeitos pós-fogo nas perdas de OC no solo por escorrência superficial. Os principais resultados apontaram para (i) uma maior quantidades de cinzas na encosta orientada a norte do que na encosta orientada a sul, enquanto que para a quantidade total de carbono orgânico (TOC) nas cinzas, estas não apresentaram diferenças; (ii) quer a perda total de sedimentos, quer a quantidade TOC do solo apresentou-se maior na encosta orientada a norte do que a sul; (iii) a fração de OC que apresentou as maiores perdas, para ambas as encostas, foi a particulada. A quantificação das perdas de OC pós-fogo podem contribuir de forma relevante para a proteção dos ecossistemas, nomeadamente em termos da fertilidade do solo.

keywords Carbon, eucalypt, forest fires, sediment losses, dissolved organic carbon, particulate organic carbon, erosion, analytical techniques, biomarkers

abstract

Soil is considered the largest carbon reservoir and an important global sink for atmospheric CO_2. Wildfires are frequent in Mediterranean ecosystems, especially in Portugal. In recent decades there has been an increase in the number of fires and climate change scenarios suggest that the fire regimes are likely to increase in the future. Forest fires can have important short−to long−term implications for key aspects of soil quality, such as the quantity and quality of soil organic matter (SOM). Due to high amount of carbon (C) stored in soil, even slight alterations of SOM can affect significantly biogeochemical cycles, hence, affecting the whole global climate. Although numerous studies have documented the effects of wildfires on hydrological and erosion processes, the effects of fire on the quantity (in terms of stocks and losses of OC content by overland flow) and quality of SOM and in the sediments eroded, as well on post-fire SOM recovery, have received considerably less research attention. These were the principal goals of the present study conducted on eucalypt plantations, one of the most fire-prone forest types in north-central Portugal.

The effects of wildfires on quality of SOM was evaluated in topsoil samples (0-2 cm) on four sampling occasions, starting immediately after the first post-fire rain till two years later. It was necessary a combination of multi-analytical techniques, such as lipid-biomarker analysis by gas chromatography-mass spectrometry (GC-MS), SOM characterization by pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) and solid state ^{13}C nuclear magnetic resonance (^{13}C NMR) spectroscopy.

Post-fire OC exports by overland flow and the contributions of the dissolved organic carbon (DOC), particulate organic carbon (POC) and dissolved inorganic carbon (DIC) fractions were measured in runoff samples collected at 1- to 2- weekly intervals during the first year after the wildfire.

The results showed that wildfire produced substantial changes in the quantity and quality of SOM. These included the thermal breakdown and cracking of *n*-alkyl compounds. Ratios of short-to-long *n*-alkanes and *n*-fatty acid methyl esters (FAMEs) increased and typical carbon number predominance indexes for *n*-alkanes (odd-to-even) and *n*-FAMEs (even-to-odd) were altered.

Furthermore, the relative abundances of certain markers, which are plant-species specific were modified, especially by decreasing terpenoids such as epiglobulol, ledol and globulol, which are characteristic of *Eucalyptus globulus*. Other differences observed in the burnt soil were the appearance of levoglucosan, a typical marker for the thermal alteration of polysaccharides, larger relative abundances of lignin-derived compounds (vanillin and methoxyphenols) and the presence of N-heteroaromatic structures. The ^{13}C NMR spectra also indicated that the wildfire produced a considerable increase in the aromaticity and aromatic condensation of the topsoil SOM. The continuation of these differences in SOM quality during the period of this study, suggested a slow recovery of soil properties, possibly influenced by a limited recovery of the vegetation after the fire combined with the fire-enhanced losses of soil.

The present work also evaluated post-fire soil OC losses by overland flow in recently two burnt eucalypt plantations, addressing a topic that has seldom been investigated. The main findings were that: (i) the amount of deposited ashes was higher at the NW slope than at the SE slope, while ashes total organic carbon (TOC) content revealed no differences; (ii) total sediment losses and also the TOC export were higher at the NW slope than at the SE slope; (iii) particulate organic carbon fraction showed the highest loss at the both topsoil sites. In addition, this study provides some insight into post-fire organic carbon losses in the recently burnt areas, which is crucial information for ecosystem management, especially in terms of soil fertility.

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Chapter 1

Introduction

1.1. Wildfires: occurrence and effects on Mediterranean ecosystems

Wildfires has been considered as one of the most severe environmental problems in the world, destroying several hundred million hectares of forest and other vegetation types every year (FAO, 2010). In the Mediterranean region, forest fires have affected large areas causing a significant negative impact on the biodiversity. Mediterranean forests, situated in a transitional zone between the European, African and Asian continents, are a center of plant diversity, with 25,000 flora species and 30,000 sub-species. This represents 10% of the world's flowering plants on just over 1.6% of the Earth's surface (FAO, 2013).

Historically, fire has been used for thousands of years as a land management tool. However, since 1960 forest fires frequency and severity has increased, affecting on average, every year 400,000 hectares of forest and other wooded land (FAO, 2013). Depending on the socio-economic context of the region concerned, variables linked to agricultural activity can explain the ignition of intentional and unintentional fires. Abiotic factors related to weather, changes in land use, which resulted in extensive land abandonment, increases in the fuel load and continuity in the landscape are the most significant environmental factors that drive ignition of forest fires (Pausas et al., 2008; Moreira et al., 2011; Ganteaume et al., 2013). Furthermore, based on climate change projections, more frequent and longer droughts will also contribute to the increase of the risk of wildfire occurrence in the Mediterranean regions (Doerr and Cerdá, 2005; Duguy et al., 2013). The intensification of forest fires, the capacity of the Mediterranean ecosystems to naturally regenerate in many areas has been reduced, while extensive areas are being affected by biodiversity loss, soil erosion and water scarcity (Shakesby, 2011; Fernandes, 2013).

Portugal is one of the Mediterranean countries most affected by forest fires, on average 100,000 ha were consumed during the past three decades (Pereira et al., 2006; ICNF, 2015). The occurrence and extent of wildfires have been increasing (Figure 1), of which 2003 and 2005 were the most dramatic years with 425,000 and 340,000 hectares burnt, respectively. In 2010, year of the beginning of this study, a total of 22,027 fire occurrences

were registered affecting 133,091 hectares (35% of forest and 65% of shrubland) (ICNF, 2010).

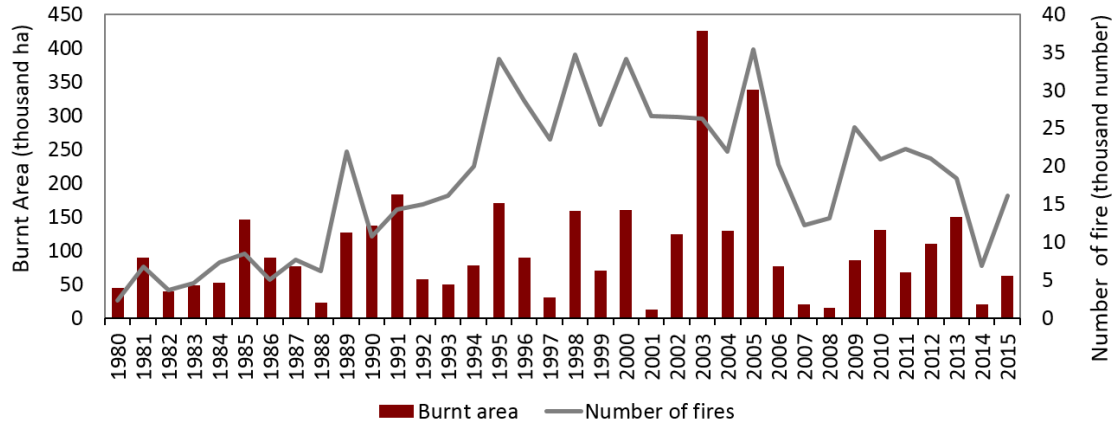

Figure 1 - Distribution of burnt area and number of forest fire during 1980-2015 (ICNF, 2015).

According to the "Inventário Florestal Nacional" (ICNF, 2013), the most part of Portugal land use is forest (35.4%), being *Eucalyptus globulus* (Labill.) and *Pinus pinaster* (Ait.) the most abundant plantations, covering 26% and 23%, respectively, of the total forest area. Both tree species are fire prone species: eucalypt because of the existence of volatile substances and pines because it accumulates a leaf litter and its high content of resin (Moreira et al., 2011).

The fire incidence and burnt area in Portugal is not expected to decline markedly in the foreseeable future. This can be due to the economic importance of the country's forestry activities, in particular with the large-scale replacement of native Portuguese forest by commercial plantations of eucalypt and the likely increase in the occurrence of meteorological conditions conducive to fire (Pereira et al., 2006; Carvalho et al., 2010).

Fires exert multiple levels of effects on the atmosphere, soil and aquatic ecosystems, which may probably cause substantial hydrological and geomorphological change on forest ecosystem processes (Shakesby and Doerr, 2006; Conard and Solomon, 2008; Mataix-Solera et al., 2011; Shakesby, 2011; Martins et al., 2012). The most important impacts on soils are the reduction of vegetation cover, which lead to increases in soil erosion rates and changes to runoff generation, the deposition of ash after combustion of biomass, the induction of enhancement of water repellency (SWR), changes in the structure and soil components, the export of pollutants, sediments, soil organic matter

(SOM), nutrients and other constituents (González-Pérez et al., 2004; Keizer et al., 2008; Smith et al., 2011; Badía-Villas et al., 2014; Faria et al., 2015; Machado et al., 2015; Malvar et al., 2015). The magnitude of these fire effects can be highly variable due to the large number of controlling factors, such as fire regime (severity, duration and recurrence), as well as local conditions, such as type of soil, vegetation composition, topography or regional climate (Neary et al., 1999; Shakesby and Doerr, 2006).

1.2. Soil Organic Matter and Soil Organic Carbon: definition, function and dynamics

Soil can be defined as a complex system, consisting of a mixture of organic and mineral particles, soil solution and air (Figure 2), resulting from the interaction between biotic and abiotic factors; it is the medium in which plants acquire water and nutrients through their root systems (Kutsch et al., 2009).

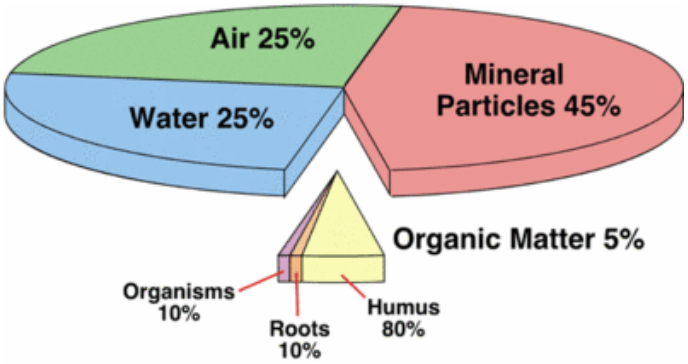

Figure 2 - Distribution of major components of soil. Organic matter can be further sub-divided into humus, roots and living organisms. The values given above are for an average soil. Source: Pidwirny (2006).

The relative proportion of these four components of soil greatly influences the physical and chemical properties of soil and depends on the amount of vegetation, soil compaction, and water present in the soil (Kutsch et al., 2009).

Soil organic matter (SOM) corresponds to all live or dead organic materials found in the soil, including plant roots, soil microorganisms and microfauna, as well as decomposed and nondecomposed plant residue. SOM also contains key elements that are essential for plant nutrition: carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). It also includes minor elements, such as sulphur (S), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and trace elements (Baldock and Skjemstad, 1999; SSS, 2008).

Soil organic carbon (SOC) represents around 50 to 60% of SOM and plays a very important function in the growth of plants through maintenance, as well as improvement of many soil properties. In terrestrial ecosystems soils represent the major reservoir of organic

carbon (OC), but with large and yet unquantified uncertainties in their estimates (mainly due to low soil sample numbers used for global upscaling and assumptions on mean soil depths). At the global level, SOC pool (estimated to 1 m depth) contains about 1500-2000 PgC (Pg=1015 g), more than twice the amount stored in the atmosphere (750 PgC). Thus, small disturbances in this SOC pool have the potential to release a large amount of C into the atmosphere (Janzen, 2004; Lal, 2004).

The content of SOC in a particular soil is determined by many factors including climatic factors (temperature and moisture regime) and soil related factors (soil parent material, clay content, cation exchange capacity) (Dawson and Smith, 2007).

The global SOC (0-20 cm) content map of Europe (Figure 3a) shows that the largest OC contents were observed in Ireland, the United Kingdom, Sweden, Finland, Estonia and Latvia, mostly in wetlands (peat lands), woodlands and in mountainous areas. In the case of the Mediterranean countries, lowest SOC concentrations were predicted. This can be a consequence of the intensive management practices, which increase the mineralisation of SOM and therefore reduce SOC contents (de Brogniez et al., 2015). Other study, conducted in Portugal by Rosário (2010) reported contents of SOC (0-40 cm) between 100 and 240 t.ha^{-1} in north-central Portugal (Figure 3b).

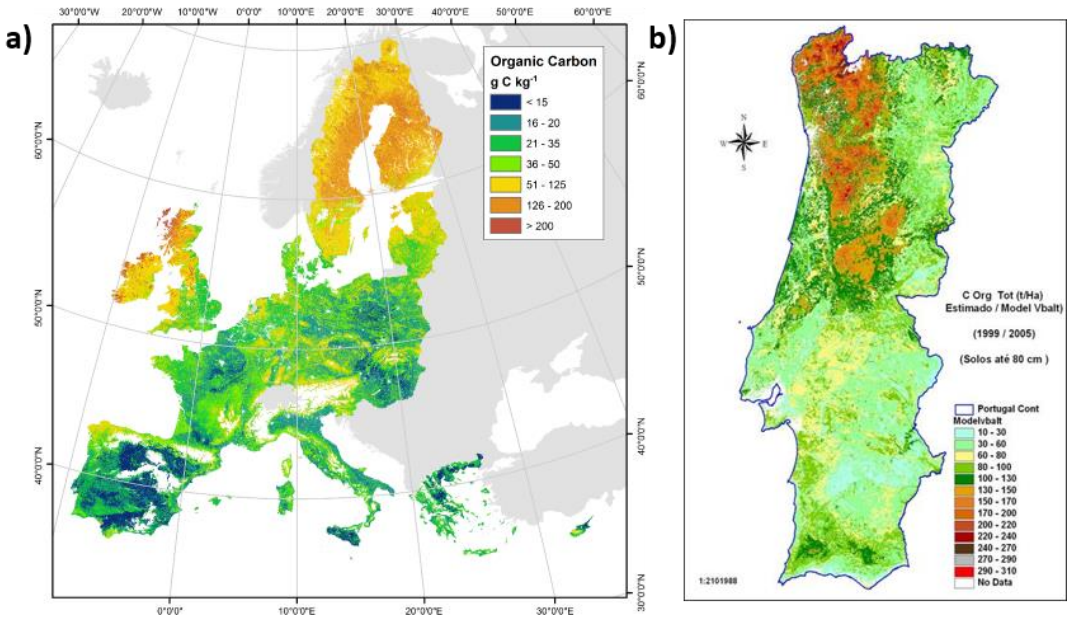

Figure 3 - SOC (0-20 cm) content (gC kg^{-1}) generated by a generalized additive model (a) and SOC (0-40 cm) in Portugal (soil sampling between 1999/2005) predicted by stepwise regression model (b). Source: de Brogniez et al. (2015) and Rosário (2010).

The dynamics of C transformations and transport in soil are complex. In natural ecosystems, losses of C from the soil derive mainly from decomposition and mineralisation processes of SOM, which lead to the release of CO_2 and some other trace gases (CH_4 and CO), or in groundwater as dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC), or export of C in various forms into aquatic systems (Figure 4). SOC accumulation, under undisturbed natural conditions, is in equilibrium and the input of C (litterfall, root biomass, etc.) is balanced by output (erosion, decomposition, and leaching) (Reichle et al., 1999; Lal, 2004).

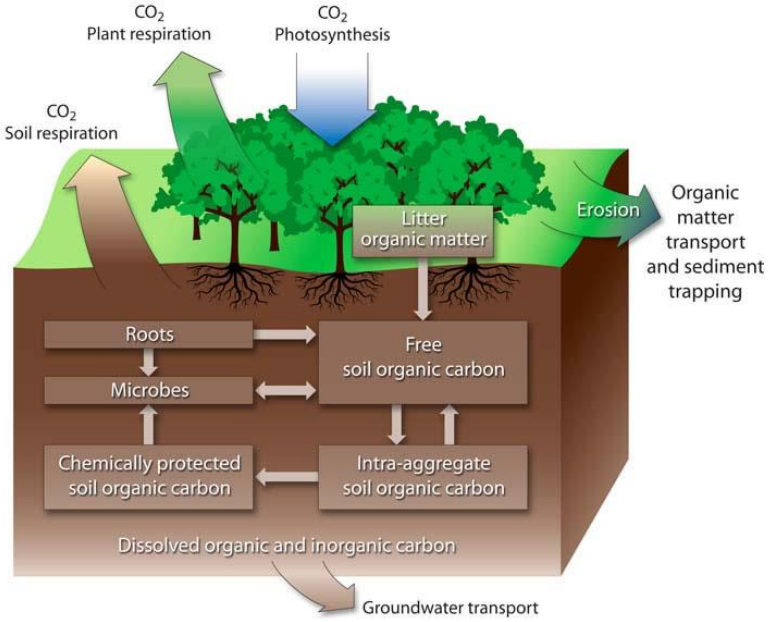

Figure 4 - Simplified pathways of C through ecosystems. Adapted from Reichle et al. (1999).

SOM has been constituted by the dissolved organic matter (DOM) and particulate organic matter (POM) (Figure 5). DOM represents one of the most mobile and reactive organic matter fractions, thereby controlling a number of physical, chemical and biological processes, in both terrestrial and aquatic environments (Bolan et al., 2011). Since C represents the bulk of the elemental composition of the SOM, DOM is often quantified by its C content and referred to as dissolved organic carbon (DOC). DOC can be further divided, based on composition, into humic and non-humic fractions. Humic material contains both aromatic and aliphatic components with amide, carboxyl, ketone, and other

functional groups (Leenheer and Croué, 2003). The humic fraction can be further categorised into humic acids, fulvic acids, and humin. The criteria for inclusion each of one in these three categories are based on solubility properties at specific pH levels (Bauer and Bianchi, 2011).

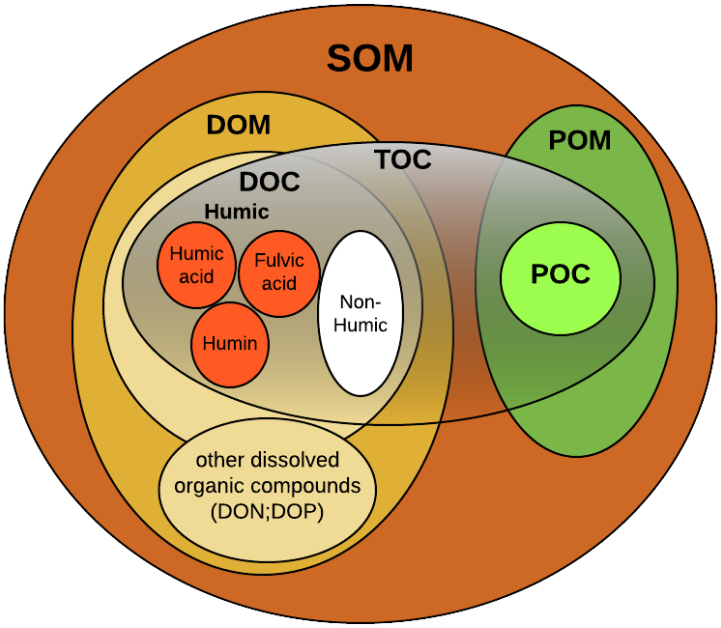

Figure 5 - Forms of soil organic matter (SOM) found in natural soil. Soil organic carbon (SOC), dissolved organic matter (DOM), total organic carbon (TOC), dissolved organic carbon (DOC), particulate organic carbon (POC), dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) are represented. DOC can be further broken down to its humic (humic acid, fulvic acid, and humin) and non-humic material. Adapted from (Pagano et al., 2014).

1.3. Fire effects on SOM and SOC

The effects of a wildfire at the soil level can be distinguished into two groups: (i) as a direct effect of high temperatures, there is an increased mineralization of SOM (with consequent reduction in structural complexity and increase in pH through the release of soluble cations); (ii) as an indirect effect resulting from the loss of vegetation and litter, there is a higher vulnerability to erosive processes plus considerable changes in the hydrological regime and sediment transport dynamics of the burnt areas (Certini, 2005).

The organic component of soil is one of the most impacted by fire, in terms of content and composition (González-Pérez et al., 2004; De la Rosa et al., 2013; Badía-Villas et al., 2014; Faria et al., 2015b). The magnitude and duration of these changes on SOM depends largely on the chemical and physical properties of the soil, the nature of the biological communities of the respective ecosystem, and the severity of the fire (Knicker, 2007). The assessment of the overall effect of fire on SOC is very complex.

Changes in SOC content range from total destruction by very intense fires to increases up to 30% (De la Rosa et al., 2013) as a consequence of external inputs, mainly from leaves and partially burnt plant materials. In addition, the effects are usually very different in the O horizon than the A horizon. With respect to the O horizon, fire can cause a significant reduction in SOC content in the short-term because of mineralisation of C from the OM, followed by volatilisation, ash removal by wind, soil erosion and surface runoff. In terms of long-term, SOC content can increase because of incorporation of the ash into soil, vegetation recovery and decomposition of partially burned woody fragments (Caon et al., 2014). Regarding to the A horizon, most of the studies reported decreases SOC content after a fire in both short- and long term for high severity fires. However, it is necessary more research about the factors affecting SOC content in the A horizon, as for example, the changes on SOM decomposition rate (Caon et al., 2014).

Considerable post-wildfire soil erosion rates have been observed in burnt Mediterranean forest. Shakesby (2011) reported soil erosion rates ranging between 0.10− 0.39 Mg ha^{-1} y^{-1} for low severity fire, 2.10−3.28 for moderate severity and 3.70−10.8 Mg ha^{-1} y^{-1} for high severity, measured on field plots in the Mediterranean areas one year after the fire.

Other studies in north-central Portugal reported soil losses of 0.6 Mg ha^{-1} y^{-1} during the first two post-fire years in a pine plantation (Martins et al., 2013) and 1.1–8 Mg ha^{-1} y^{-1} in eucalypt plantations during the first year after fire (Prats et al., 2014; Faria et al., 2015a).

The loss of soils through erosion and overland flow can represent an important pathway of SOC loss, either in dissolved or in particulate form. In addition, to the post-fire SOC exports and its significant impacts on-site soil degradation as well as on the global C cycle, there are also important effects for the aquatic ecosystems (Smith et al., 2011). However, the post-fire SOC impacts have been poorly studied in the Mediterranean region and, in particular in Portugal. Shakesby et al. (2013) reported modest fire effects on soil quality, since the total estimated post-prescribed fire particulate losses of OM represented only 0.5–2.9% of the content in the upper 2 cm of soil. Therefore is a need to investigate the post-fire effects on SOC export behavior (both dissolved and particulate forms).

Alterations in SOM quality typically consist of an increase in aromatic C through charring at the expense of a decrease in thermo-labile O-alkyl C, and of a conversion of peptide N into heterocyclic structures (Knicker et al., 2006). While such marked re-arrangements of C forms by heating can include specific aspects such as, for example, the formation of new water-repellent substances (Atanassova & Doerr, 2011; de Blas et al., 2013), it tends to affect soil health and quality as a whole, including biodiversity and biological activity (González-Pérez et al., 2004). In many cases, however, fire effects on SOM are thought to be reversible, reverting naturally to (almost) pre-fire conditions (González-Pérez et al., 2008).

The effects of fire on SOM quality continue an important research gap, also because the improvement of analytical techniques useful for a better knowledge about SOM composition at molecular level, such as pyrolysis gas chromatography/mass spectrometry (Py–GC/MS) and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, have shown tend to be very complex (De la Rosa et al., 2012; Faria et al., 2015). Hence, a better understanding of the overall wildfires effects on SOM is required for a more accurate assessment of the risks of post-fire soil fertility and its possible impacts on forest recovery.

1.4. Objectives and thesis structure

The overall aim of this study was to further knowledge and understanding of the effects (direct and indirect) of wildfire on the quantity (in terms of stocks and losses of SOC content by overland flow) and quality of SOM in burnt forest areas in north-central Portugal. In order to evaluate the alterations on the SOM quality, it was necessary to apply a combination of sophisticated analytical techniques such as lipid-biomarkers analysis by gas chromatography-mass spectrometry (GC-MS), pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS), and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

The specific objectives were the following:

i. To assess the performance of the analytical pyrolysis technique (Py-GC/MS) to study the wildfire-induced changes on SOM composition for distinct forest types (pine and eucalypt) one year after the fire (**Article I**);

ii. To determine the effects of wildfire on *in-situ* SOM quality by comparing soil samples collected 1, 6, 12 and 18 months after the wildfire at two neighbouring burnt/long-unburnt eucalypt plantations and their post-fire recovery of its lipid composition (**Article II**);

iii. To evaluate the influence of time-since-fire on the molecular composition of SOM in the topsoil and eroded sediments by post-fire overland flow from the same eucalypt plantations (**Article III**);

iv. To assess the effects of wildfire on carbon stocks in ashes and topsoil and their export by overland flow at the micro-plot scale during the first hydrologic year. Furthermore, the temporal patterns of both dissolved and particulate fractions were also quantified (**Article IV**).

The framework in which these studies were developed varied between the individual research grant attributed to the author (SFRH/BD/75562/2010) by the Foundation for Science and Technology (FCT) of Portugal as well as by the FIRECNUTS project (PTDC/AGR-CFL/104559/2008) funded by FEDER through the POCI2010 Programme. The application of the analytical techniques described previously has been made possible through a close cooperation with *Instituto de Recursos Naturales y Agrobiología de Seville* (IRNAS-CSIC) team, which permitted the fellow not only having access to the facilities but also a complete training in the use of such equipment. On the other hand, the training awards supported by the programmes "Natural molecular structures as drivers and tracers of terrestrial C fluxes" (MOLTER) and Natural Organic Matter Research – International Humic Substances Society (IHSS), respectively funded the author stay at IRNAS-CSIC.

The structure of this thesis follows four main chapters. The first chapter comprises a general introduction and chapter 2 corresponds to the publications in which objectives of the present work were set. Chapter 3 mentions other contributions by the author. Aftewards, the thesis is followed by general conclusions and future perspectives (Chapter 4).

Chapter 2

Direct and indirect wildfire effects on SOM/SOC

2.1. Characterization of wildfire effects on soil organic matter using analytical pyrolysis (Article I)

This chapter has been published in:

*De la Rosa, J.M., **Faria, S.R.**, Varela, M.E., Knicker, H., González-Vila, F.J., González-Pérez, J.A., Keizer, J.J., 2012. Characterization of wildfire effects on soil organic matter using analytical pyrolysis. Geoderma, 191, 24-30.*

Abstract

The effectiveness of pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) as a rapid analytical technique to get insight in wildfire-induced molecular alterations of the soil organic matter in Mediterranean Leptosols was examined. To this end, the topsoil of two slopes with adjacent patches of burnt and unburnt Maritime Pine (*Pinus pinaster*) and eucalypt plantations (*Eucalyptus globulus*) were sampled in the Serra de Lousã, central Portugal. Several differences were found between the neighbouring burnt and unburnt soils, both in thermal desorption and pyrolysis behaviour. Thermal desorption results showed large amounts of aliphatic compounds (both alkyl and carbohydrate-derived compounds), which may indicate the incorporation of fresh plant material or low wildfire severity. Pyrolysis at 500 °C revealed an increase of low molecular weight molecules for certain homologous series in fire-affected soils, suggesting the occurrence of thermal breakdown and cracking of long-chain components. In addition, the presence of several thermo-labile markers pointed to the low severity of the wildfire. Elemental analysis indicated marked fire-induced increases in TOC and TN for the pine stand as opposed to noticeable decreases for the eucalypt stands. Probably, this contrast between the two sites is not due to differences in direct fire effects (especially fire severity), but to indirect fire effects, i.e. in particular needle/leaf fall from affected canopies.

Introduction

Wildfires are a frequent phenomenon in Mediterranean ecosystems, and are widely considered to be the main factor of disturbance in the Mediterranean basin. The Iberian Peninsula has the highest risk of wildfire occurrence of Europe and, in the case of Portugal, wildfires have during the past three decades devastated over 100,000 ha each year, with dramatically higher figures for dry years like 2003 and 2005 (Pereira et al., 2005). Amongst the panoply of wildfire-induced changes in soil physical, chemical, mineralogical and biological properties, the most commonly reported changes are those in soil organic matter (SOM), both in terms of SOM quantity and quality (González-Pérez et al., 2004; Knicker, 2007; Varela et al., 2010).

It is widely recognised that the fire-induced changes in SOM depend to a large extent on the temperature ranges reached at different soil depths (severity) and the degree of heating that the different organic compounds can withstand before being altered (resilience). SOM is a heterogeneous and chemically-complex soil component that includes a variety of organic forms ranging from labile to refractory. Wildfires, however, can also affect SOM indirectly, e.g. through the changes in vegetation cover and in geomorphological processes (Shakesby and Doerr, 2006).

As a direct consequence of fire, not only new forms of C and N compounds, including water-repellent substances, are produced but also previously existing C compounds are modified (Knicker et al., 2005). These changes lead to the formation of substances with weak colloidal properties and enhanced resistance to chemical and biological degradation (Almendros et al., 1992; Doerr et al., 2000; Knicker et al., 2005). The soil lipid composition is particularly prone to undergo rapid and significant alterations during a wildfire, since it is a markedly labile fraction of SOM.

Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) is a fast and reproducible technique that is widely used in the structural characterization of SOM. Py-GC/MS involves a thermolytic degradation of macromolecules into small fragments that are amenable by gas chromatography-mass spectrometry (GC/MS). It provides information concerning the structure of organic molecules, including N compounds that cannot be released by conventional hydrolysis. It is particularly useful for the direct study of

materials that, owing to their complexity, are difficult to analyze by way of conventional methods. In addition, pyrolysis of SOM generates a wide range of products that can be related to their origin, e.g. methoxyphenols from lignin, furans from polysaccharides, and N-containing molecules from proteins (Leinweber and Schulten, 1995; González-Vila et al., 2001).

In spite of the above-mentioned advantages of Py-GC/MS, only few studies (Knicker et al., 2005; Tinoco et al., 2006; De la Rosa et al., 2008) have explored this technique for studying wildfire-induced changes in SOM. The present work is the first instance in which Py-GC/MS is applied for a comparative study of wildfire effects for distinct forest types, focussing especially on the role of possible differences in fire severity. To this end, the topsoil of adjacent burnt and unburnt patches of a maritime pine stand and of a eucalypt plantation was analysed in terms of SOM quality as well as elemental composition and selected physical properties. For the characterization of the SOM quality, a double-shot pyrolysis experiment was designed in which GC/MS is used to analyze the evolved gases released in two steps, i.e. a first thermal desorption step at sub-pyrolysis temperature (300 ºC) followed by a second step at real pyrolysis temperature (500 ºC). The first step provides information about the fire-induced changes in the more labile SOM fraction, whereas the second step provides information about the changes in the compounds with higher thermal stability (Quenéa et al., 2005). The importance of distinguishing these two fractions in the present context was shown by Knicker et al. (2005), revealing that wildfires lead to substantial losses in the labile SOM constituents in particular.

Materials and Methods

Study area and field sampling

The study area is located near Colmeal village, on the border of the municipalities of Góis and Arganil, in the Coimbra District of north-central Portugal (Figure 6; 40º 08' 46'' N; 7º 59' 35'' W; 500 m asl). The climate of the study area is of a transitional Atlantic-Mediterranean type, with wet winter and dry summer. According to the available information (in map format: APA, 2011), the mean annual temperature is between 10 and 12.5 °C, and the average annual rainfall is between 1,400 and 1,600 mm.

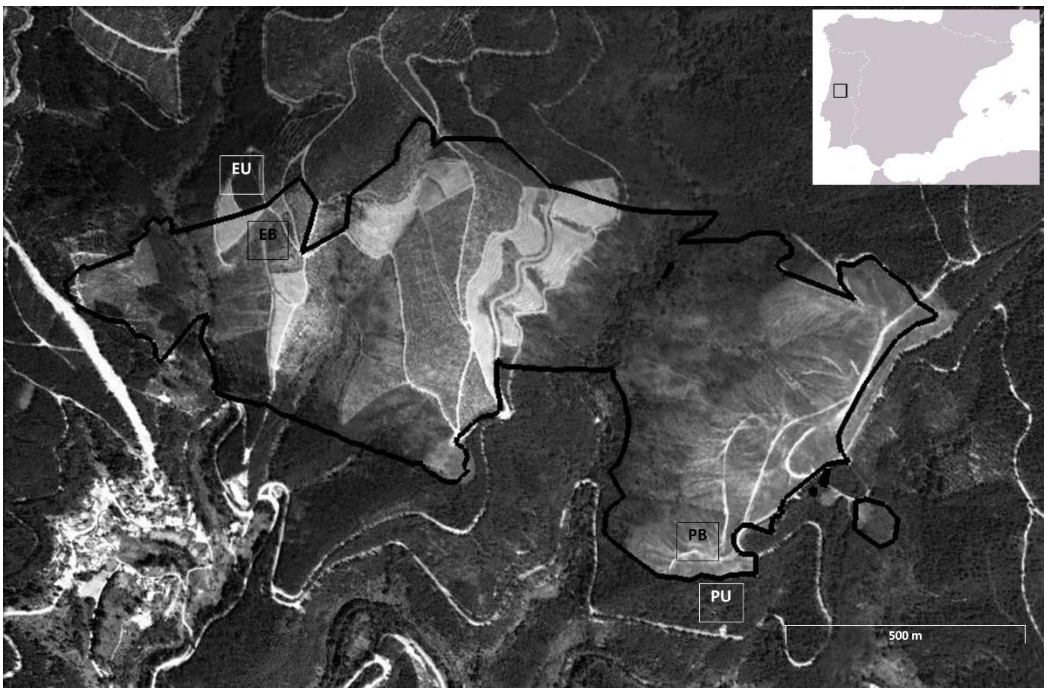

Figure 6 - Colmeal burnt area, and the burnt and unburnt Maritime Pine and eucalypt plantations (PB, PU, EB and EU, respectively).

An area of ca.70 ha, mainly consisting of maritime pine (*Pinus pinaster* Ait.) and eucalypt (*Eucalyptus globulus* Labill.) plantations, was consumed by a wildfire that started on August, 24th 2008. Following the wildfire, five hill slopes were selected for measuring soil erosion (Fernandes et al., 2010), whilst two additional hill slopes were selected for monitoring soil properties using destructive sampling techniques. The two latter sites were comprised of a maritime pine (P) and a eucalypt (E) plantation located near the limits of the burnt area, allowing us to establish neighbouring pairs of burnt and unburnt

sampling locations (PB/PU and EB/EU, respectively) in one and the same forest stand but at either side of the burnt area limit.

The eucalypt stand was planted some two years before the wildfire and terraced prior to planting, whilst the pine stand was c. 30 years old. Fire damage to the crowns of the eucalypt as well as pine trees mainly involved scorching, indicating that fire severity had been comparatively low (e.g. Shakesby and Doerr, 2006; Keizer et al., 2008).

For this study, one soil sample was collected at each of the four sample locations at 0-5 cm depth during June 2009. Following field collection, the soil samples were air-dried at ambient temperature, passed through a sieve with a mesh width of 2 mm, and stored in plastic bags at 4 ºC until the laboratory analyses were carried out in November/December 2010. Prior to elemental analysis, samples were pulverized (<0.05 mm).

Soil profiles excavated at the base of the two study sites (in the case of the eucalypt stand, a part of the slope that had not been terraced) corresponded to Umbric or Haplic Leptosols (WRB, 2006), depending on A horizon thickness, and were overlying pre-Ordovician schists of the Hercynian Massif (Ferreira, 1978).

Laboratory analyses

Total C and N concentrations (hereafter TC and TN, respectively) were determined in triplicate by the dry combustion method (975 ºC) using an Elementar Vario EL microanalyzer (Elementar Analysen systeme GmbH, Hanau, Germany) detecting N as N_2 and C as CO_2. Total Organic Carbon (TOC) was measured after removal of carbonates with 1M HCl. Briefly, 2 g of soil was treated with 25 ml of 1 M HCl (3x). The acid–soil mixture was separated by centrifugation (5000 rpm, 10 min.). Finally, soils were washed with deionised water 5 times, oven-dried at 50 °C for 72 h, and finely ground by mortar and pestle. The coefficient of variation of replicated analyses was below 5%.

Soil pH in water (soil-water ratio 1:2.5) and electrical conductivity were measured with a portable pH/EC meter (HI 991300, Hanna Instruments). The particle size distribution was determined using standard methods described by Guitián and Carballas (1976), and texture was classified according to the USDA criteria (SSS, 1975).

Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i) attached to a GC/MS system

Agilent 6890N. Soil samples 1-2 mg in weight were placed in small crucible capsules. The sample capsule was introduced in the furnace preheated at 100 °C. For the desorption step the temperature was raised to 300 °C at a rate of 20 °C min^{-1} and held at 300 °C for 1 min and the evolved gasses directly injected in the GC/MS for analysis. For the subsequent pyrolysis the temperature was held at 40 ºC for 1 min and then increased up to 100 ºC at 30 ºC min^{-1}, from 100 to 500 ºC at 20 ºC min^{-1} and isothermal at 500 ºC for 2 min and the evolved gasses injected in the GC/MS for analysis. The GC was equipped with a fused silica capillary column DB5 MS (J&W Scientific) (30 m × 250 µm × 0.25 µm film thickness), oven temperature was held at 50 ºC for 1 min and then increased up to 100 ºC at 30 ºC min^{-1}, from 100 to 300 ºC at 10 ºC min^{-1} and isothermal at 300 ºC for 10 min using a heating rate of 20 ºC min^{-1} in the scan modus. The carrier gas used was helium with a controlled flow of 1 mL min^{-1}. The detector consisted of an Agilent 5973 mass selective detector and mass spectra were acquired with a 70 eV ionizing energy.

Compound assignment was achieved via single ion monitoring for different homologous series, low resolution MS and comparison with published and stored (NIST and Wiley libraries) data. The relative abundance of compounds within the pyrolysates was calculated by normalizing individual peak area to the total peak area of all the assigned pyrolysis products i.e. the sum of all assigned pyrolysis products was defined as 100%, from which the relative contribution of each was calculated.

Results and discussion

Chemical characteristics

The elemental composition (TN, TC, TOC, TOC/N) as well as the selected physico-chemical characteristics of the studied soil samples are shown in Table 1. In the case of the pine stand soils, TN, TOC and TOC/TN-ratio were higher for the burnt than unburnt sample, whereas the opposite was true in the case of the eucalypt stand.

Table 1 - Elemental analysis (TN, TC, TOC), pH, cation exchange capacity and texture of soil samples.

Sample	Code	TN (%)	TC (%)	TOC (%)	TOC/N	pH (in H_20)	CEC (meq 100 g^{-1})	Texture
Pine unburnt	PC	0.45	9.6	7.8	17.3	4.73	47	Sandy loam
Pine burnt	PB	0.50	10.6	10.2	20.4	4.30	60	Sandy clay loam
Eucalypt unburnt	EC	0.44	8.2	7.7	17.5	4.54	48	Sandy clay loam
Eucalypt burnt	EB	0.29	4.3	3.9	13.4	4.94	43	Sandy loam

Total OC and TN contents are well-known to be highly variable, depending on key factors such as fire type (canopy or aboveground, underground fires), fire severity, vegetation cover and terrain (Chandler et al., 1983; Fernández et al., 1997; Kavdir et al., 2005; Tinoco et al., 2006; De la Rosa et al., 2009). A contrast between the pine and eucalypt plantation also existed for pH and cation exchange capacity (CEC), although the number of samples analysed here makes any interpretation highly speculative.

Py-GC/MS

The total ion current (TIC) traces and released compounds obtained by GC/MS analysis of the two successive shots are reported in Figures 7 and 8, and Tables 2 and 3, respectively. Conspicuous differences in composition between the first and the second shot were found.

First Shot (Desorption at 300 ºC)

Clear differences were found when comparing the total ion chromatograms (TIC) of the samples at 300 ºC (desorption step). In general the TICs were dominated by furfural, furanmethanol (PS1 and PS3) and other compounds of carbohydrate origin (PS) (Table 2; Fig 7). These compounds are considered as thermally labile. Therefore, their presence in the burnt soil samples (PB and EB), albeit with a lower relative abundance than in the unburnt samples (PU and EU; Fig 7), suggests a low-severity wildfire. In addition, a series of *n*-alkane/enes (from C_{10} to C_{29}), aromatic compounds derived from methyl-benzene and naphthalene (A1 to A8), and N-containing products from peptides (P1 to P4) were detected in most samples. However, long-chain *n*-alkanes, substituted-Naphthalenes and some peptides-derived compounds (especially indoles and indenes) were not detected in the burnt soils (samples PB and EB), whilst they were found in the unburnt soil samples. This clearly points to heat-induced alterations of the more labile OM weakly bound to soil matrix.

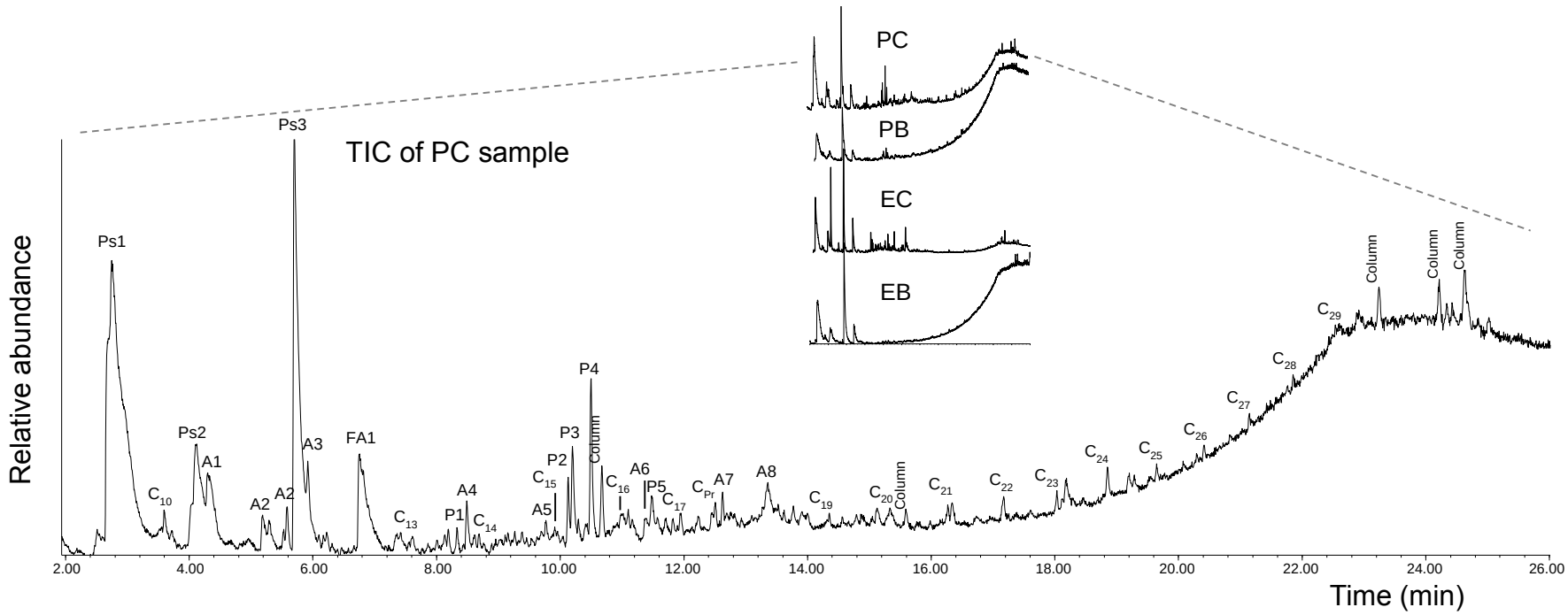

Figure 7 - Py-GC/MS total ion chromatogram of burnt and unburnt Mediterranean soils under pine and eucalypt plantations (PB, PU, EB and EU respectively) at 300 °C. See Table 2 for the identification of the peak labels.

Table 2 - Py-GC/MS compounds released at 300 ºC. (Desorption step)

Peak label	Assignment	Origin	PC	PB	EC	EB
A1	toluene	Ar	x	x	x	x
A2	benzene, 1,4-diethyl	Ar	x		x	
A3	isobenzene	Ar	x		x	
A4	naphthalene, x,x-dimethyl	Ar	x		x	
A5	naphthalene, x,x,x-trimethyl	Ar	x		x	
A6	naphthalene, x,x,x,x-tretramethyl	Ar	x		x	
A7	naphthalene, x,x-dimethyl-x-isopropyl	Ar	x		x	
A8	naphthalene, x,x,x,x,x-pentamethyl	Ar	x		x	
C_{10}	decane	Alk	x	x	x	x
C_{15}	pentadecane	Alk	x		x	
C_{17}	heptadecane	Alk	x	x	x	
C_{19}	nonadecane	Alk	x		x	x
C_{20}	eicosane	Alk	x	x	x	x
C_{21}	heneicosane	Alk	x	x	x	x
C_{22}	docosane	Alk	x	x	x	x
C_{23}	tricosane	Alk	x	x	x	x
C_{24}	tetracosane	Alk	x	x	x	
C_{25}	pentacosane	Alk	x	x	x	x
C_{26}	hexacosane	Alk	x		x	
C_{27}	heptacosane	Alk	x		x	
C_{28}	octacosane	Alk	x		x	
C_{29}	nonacosane	Alk	x		x	
C_{Pr}	pristane	Alk*	x		x	
FA1	butanoic acid, 2-methyl	FA	x	x	x	x
P1	1-H-indole	P	x		x	
P2	1-H-inden-1-one-x,x-dimethyl	P	x	x	x	
P3	1-H-indane-x,x,x-trimethyl	P	x	x	x	
P4	dihydroindene-x,x,x-trimethyl	P	x	x		
P5	substituted-indene	P	x		x	
PS1	furfural	PS	x	x	x	x
PS2	2-furancarboxaldehyde	PS	x		x	
PS3	2-furanmethanol	PS	x	x	x	x

Alk, Ar, PS, P and FA codes refer to molecules derived from *n*-alkanes/enes, aromatic compounds, polysaccharides, peptides and fatty acids, respectively. C_{Pr}; Pristane.* Terpenoid alkane.

Second Shot (Pyrolysis at 500 ºC)

Pyrolysis at 500 ºC presented no qualitative differences between the burnt and unburnt samples (Fig. 8). The TIC traces were characterized by the presence of complex mixtures of compounds that are typical pyrolitic products from SOM, i.e.: (i) *n*-alkanes/enes pairs (pattern from C_{10} to C_{33}); (ii) aromatic structures, mainly composed of benzenes and naphthalenes (A1 to A26; Table 3); (iii) polysaccharides-derived compounds, mostly comprising furanes (Ps1 to Ps7); (iv) N-containing products derived from peptides (P1 to P4). At lower relative abundances, the TIC traces also indicated the presence of: (i) fatty acid methyl esters (FAMEs); (ii) *n*-alkyl ketones (K); (iii) *n*-alkane nitriles (AN); (iv) lignin-derived products; (v) triterpenoids and sterols that are possible derived from plant material (St1-St3; Table 3). Since the bulk of these compounds have a unique and known source, these can be used as “fingerprint” for detecting differences and changes in SOM composition (Almendros et al., 1997).

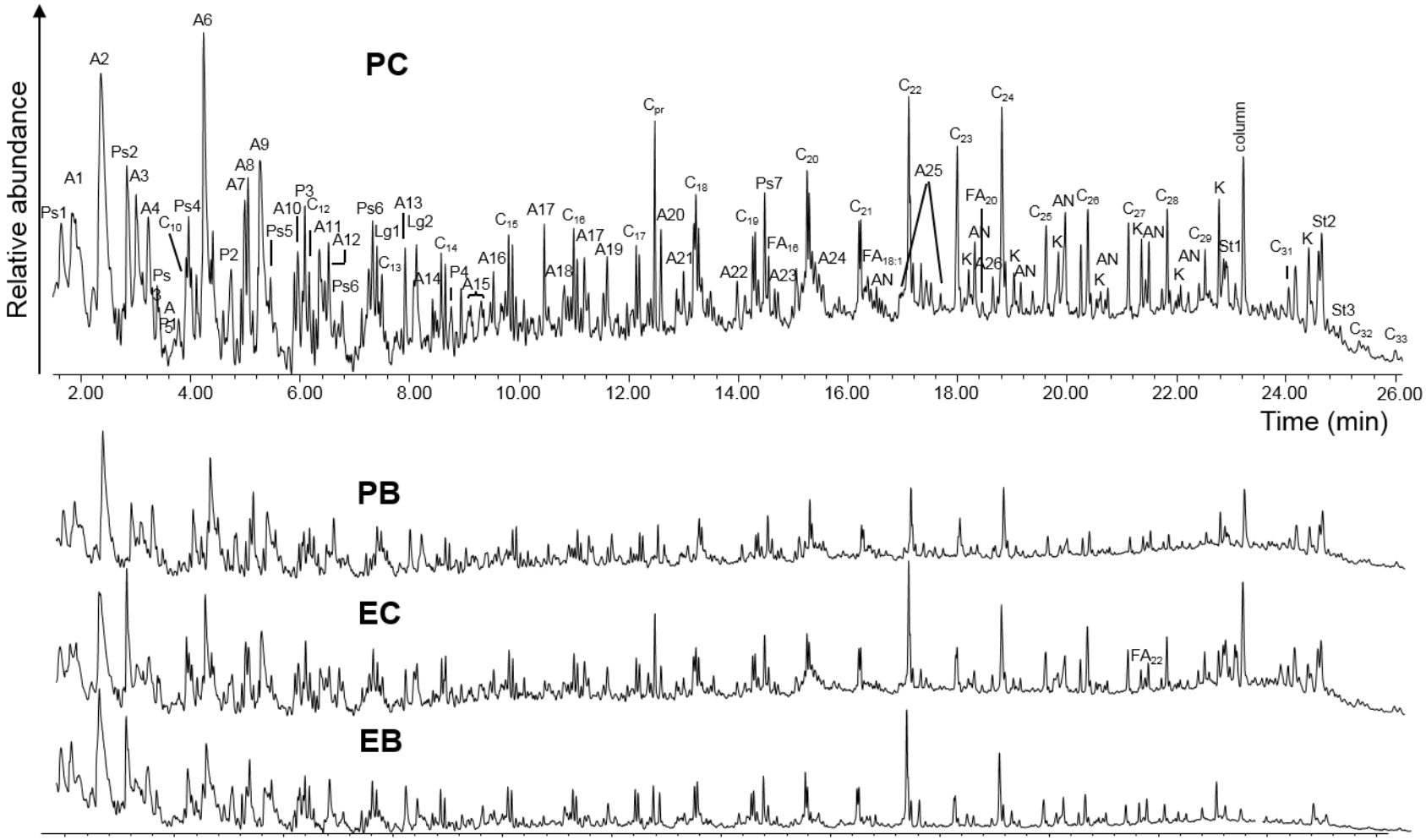

Figure 8 - Py-GC/MS total ion chromatogram of burnt and unburnt Mediterranean soils under pine and eucalypt plantations (PB, PU, EB and EU respectively) at 500 °C. See Table 3 for the identification of the peak labels.

Table 3 - Py-GC/MS compounds released at 500 ºC. (Pyrolysis step)

Peak label	Assignment	Origin
A1	benzene	Ar
A2	toluene	Ar
A3	benzene, 1,3-dimethyl	Ar
A4	Styrene	Ar
A5	benzene, x,x,x-trimethyl	Ar
A6	Phenol	Ar
A7	phenol, 2-methyl	Ar
A8	acetophenone	Ar
A9	phenol, 3-methyl	Ar
A10	benzene, 1,2,4,5-tetramethyl	Ar
A11	phenol, x-ethyl	Ar
A12	naphthalene	Ar
A13	naphthalene, 2-methyl	Ar
A14	benzene,heptil	Ar
A15	1,1-biphenyl	Ar
A16	naphthalene, x,x-dimethyl	Ar
A17	naphthalene, x,x,x-trimethyl	Ar
A18	benzene, nonyl	Ar
A19	Fluorene	Ar
A20	9H-fluorene, x-methyl	Ar
A21	naphthalene, x-isopropyl-x-methyl	Ar
A22	phenanthrene	Ar
A23	*n*-alkylbenzene	Ar
A24	anthracene, x-methyl	Ar
A25	phenanthrene, x-methyl	Ar
A26	phenanthrene, x,x-dimethyl	Ar
An	alkanenitrile	AN
C_{10}	decane/ene pair	Alk
C_{12}	dodecane/ene pair	Alk
C_{14}	tetradecane/ene pair	Alk
C_{15}	pentadecane/ene pair	Alk
C_{16}	hexadecane/ene pair	Alk
C_{17}	heptadecane/ene pair	Alk
C_{18}	octadecane/ene pair	Alk
C_{19}	nonadecane/ene pair	Alk
C_{20}	eicosane/ene pair	Alk
C_{21}	heneicosane/ene pair	Alk
C_{22}	docosane/ene pair	Alk
C_{23}	tricosane/ene pair	Alk
C_{24}	tetracosane/ene pair	Alk
C_{25}	pentacosane/ene pair	Alk
C_{26}	hexacosane/ene pair	Alk
C_{27}	heptacosane/ene pair	Alk
C_{28}	octacosane/ene pair	Alk

(Continuation)

Peak label	Assignment	Origin
C_{29}	nonacosane/ene pair	Alk
C_{30}	triacontane/ene pair	Alk
C_{31}	henetriacontane/ene pair	Alk
C_{32}	dotriacontane/ene pair	Alk
C_{33}	tritriacontane/ene pair	Alk
C_{Pr}	Pristane	Alk*
FA_{16}	hexadecanoic acid, methyl ester	FAME
$FA_{18:1}$	octadec-1-en-oic acid, methyl ester	FAME
FA_{20}	eicosanoic acid methyl ester	FAME
FA_{22}	docosanoic acid, methyl ester	FAME
K	*n*-alkyl ketone	K
Lg1	Ethylguaiacol	Lg
Lg2	2-methoxy-4-vinylphenol	Lg
St1	neoursa-3,12-diene	St
St2	β-sitosterol	St
St3	methyl substituted sterol	St
P1	pyridine, 3-methyl	P
P2	1H-indene	P
P3	1H-indene, 1-methyl	P
P4	1H-indane, 1,2,3-trimethyl	P
Ps1	furan, 2-methyl	PS
Ps2	furfural	PS
Ps3	2-cyclopenten-1-one-2-methyl	PS
Ps4	2-furancarboxaldehyde, 5-methyl	PS
Ps5	benzofuran, 2-methyl	PS
Ps6	benzofuran, x,x-dimethyl	PS
Ps7	furan, *n*-alkyl	PS

Alk, Ar, PS, P, Lg, FAME, AN, K and St codes refer to molecules derived from n-alkanes/enes, aromatic compounds, polysaccharides, peptides, lignin, fatty acids methyl esters, *n*-alkyl nitriles, *n*-alkyl ketones and steroids, respectively. C_{Pr}; Pristane.* Terpenoid alkane.

Phenol (A6) and methyl-phenols (A9, A7) were amongst the most abundant compounds in all the pyrograms. They are often recognized in pyrolysates of lignin-containing tissues, and may also indicate demethylated units from microbial degradation of lignin (Saiz-Jimenez and de Leeuw, 1986) or, alternatively, correspond to secondary reaction products from thermal degradation during lignin pyrolysis (Saiz-Jimenez, 1994). Methoxyphenol

structures are known lignin-derived compounds that were detected in all the pyrograms (Lg1 and Lg2). Polysaccharides (Ps), which are ubiquitous compounds in the pyrolysis of plants and soil OM (Templier et al., 2005), are labile compounds easily altered thermally or biodegraded during the initial phases of diagenesis. Their presence, together with that of lignin derived compounds in the pyrolysates, in the burnt as well as unburnt samples suggests that part of the SOM derive directly from plant lignocellulosic materials and/or that the wildfire was of comparatively low severity.

The burnt samples presented a slight greater relative abundance of highly condensed aromatic structures (A20-A26) than the unburnt samples. These compounds are usually released during incomplete combustion processes as may occurs during wildfires and indicate the presence of char or a thermally-altered refractory OM (Tinoco et al., 2006).

The N-containing compounds were dominated by indanes (P2, P3 and P4) and *n*-alkanenitriles (AN). The indanes are probably of protein origin, reflecting microbial input but recent studies indicated that indoles or methylpyridines (P1) are also present in fresh plant material (Buurman et al., 2009). The *n*-alkanenitriles, on the other hand, could stem from the reaction of carboxylic acids and ammonia liberated from minerals during the pyrolysis process (Evans et al., 1985).

n-alkanones (K) were also present in the pyrolysates. These compounds are usually present in plants but may also derive from the thermal alteration processes and bacterial degradation of alcohols and aldehydes.

Although steroids (St) are difficult to identify by pyrolysis, the triterpenoid compound neoursa-3,12-diene (St1) was identified in all four pyrograms. Triterpenes are key biomarker components of gums and mucilages from angiosperms and, in particular, grasses like ryegrass. β-Sitosterol and x-substituted sterols, (St2 and St3 in Fig. 3) were also identified in all the samples but occurred with greater relative abundances in the unburnt than burnt soils. These compounds are well-known as stemming from vascular plants and, in particular, plant lipid membranes and waxes. Nonetheless, algae and especially fungi can also produce these compounds in soils (Weete, 1976).

The relative abundances of fatty acids and *n*-alkanes/*n*-alkenes pairs in the burnt and unburnt samples are compared in Figure 9. González-Pérez et al. (2008) found these

compounds to be particularly useful as molecular markers for detecting environmental changes including fire in soils The *n*-alkanes ranged from C_{10} to C_{33}, and revealed bimodal distributions with different maxima for the eucalypt (C_{13} and C_{21}) and pine samples (C_{15} and C_{22}). The Carbon Preference Index (CPI) of the four samples was around 0.7, confirming that there was no marked odd/even carbon number predominance as would have been expected for samples from direct plant origin. The TICs of all four samples revealed series of *n*-alkenes, primarily as terminal olefins (*n*-alk-1-enes and ranging from C_{10} to C_{32} with a maximum at C_{22}).

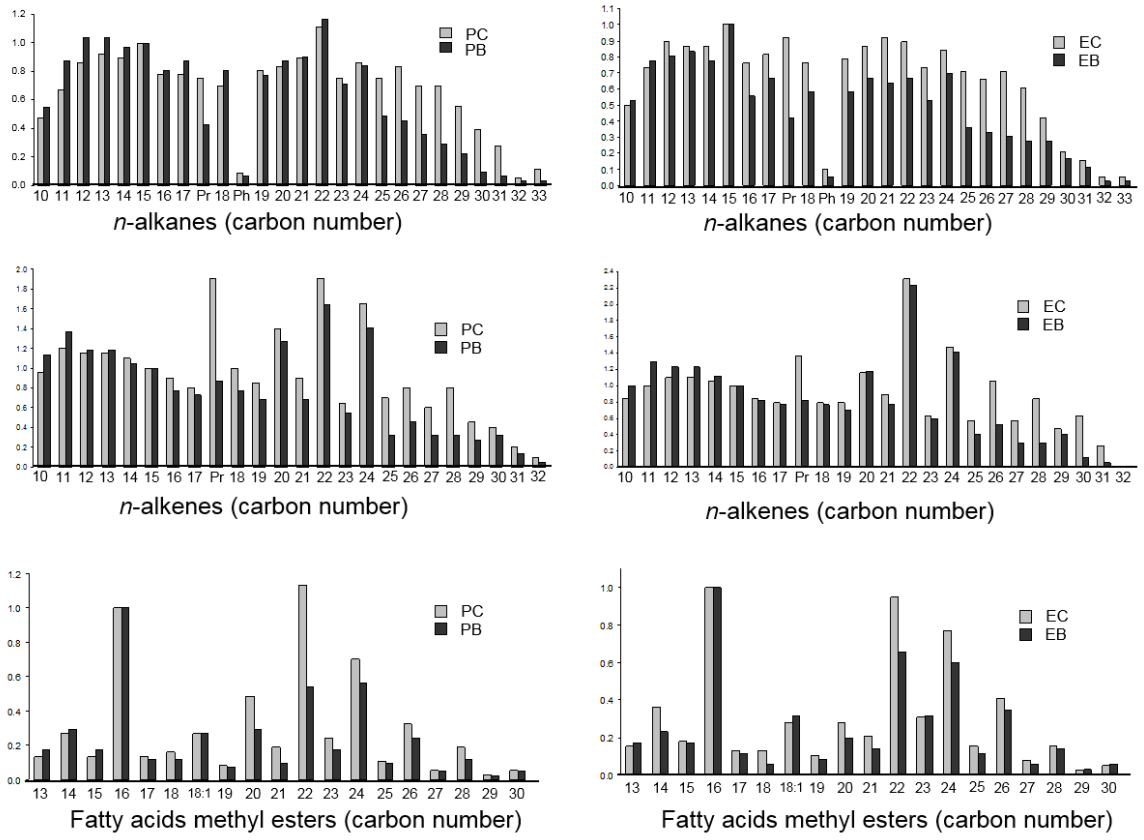

Figure 9 - Relative abundance (vertical) vs. carbon distribution (horizontal) of *n*-alkanes, *n*-alkenes and fatty acids (as methyl esters) after pyrolysis at 500 °C of bulk soils. The abundances of *n*-alkanes and *n*-alkenes are normalized to C_{15}. Abundances of fatty acids are normalized to Palmitic acid (C_{16}). Axis numeral indicates the carbon number). Pr, pristane; Ph, phytane.

A series of *n*-fatty acids (FAs) was present in the range C_{13}-C_{30}. However, analytical pyrolysis presents major limitations in the detection of FAs, due to the incompatibility of free FA with the polar columns that are typically used in GC as well as to secondary reactions such as decarboxylation occurring at elevated temperatures (Dignac et al.,

2006). As a consequence, the relative abundances of FAs were lower than those of the *n*-alkanes as well as *n*-alkenes (Fig. 3). The detected FAs revealed a predominance of even-numbered C-chains, with palmitic (C_{16}) and behenic acid (C_{22}) being the most abundant. Both palmitic and behenic compounds have been assigned to algal and bacterial OM (Kruge and Permanyer, 2004).

The relative abundances of *n*-alkanes, *n*-alkenes and *n*-fatty acids are summarised in Table 4 by means of descriptors that were used in earlier studies as markers of fire-induced changes in SOM of the subsequent recovery of SOM with time since fire (Almendros et al., 1988; Knicker et al., 2005; González-Pérez et al., 2008; Kuhn et al., 2010).

A comparable fire-induced decrease in the average chain length (ACL) of *n*-alkanes as well as *n*-alkenes was observed for both types of soils (*n*-alkanes: pine site 19.5-18.4; eucalypt site 20.0-18.4. *n*-alkenes: pine site 19.3-18.2; eucalypt site 19.7-18.3). These decreases in ACL fitted in well with the observed increases in the ratios of short-to-long *n*-alkanes at both sites (pine site: from 2.6 to 3.7; eucalypt site: from 2.2 to 4.3). The same applied, mutatis mutandis, in relation to the decreases in the ratios of long-to-total *n*-alkanes (pine site: from 0.30 to 0.18; eucalypt site: from 0.26 to 0.20). The *n*-alkenes presented similar fire-induced changes in their short-to-long and long-to-total ratios than *n*-alkanes. Thus, *n*-alkanes/-enes consistently pointed towards fire-induced breakdown of the SOM (Almendros et al., 1988), which leads to the cracking of long-chain components and the accumulation of low-molecular weight homologues in burnt soils.

The fatty acid (FA) distribution has been also used to inform about soil status and recovery after fire events (Gonzalez Vila et al., 2001). Figure 9 shows a decrease in the relative abundance of FA in the burnt compared to the unburnt soils. In addition, the increases in ratio of short chain/long chain FAs between burnt and unburnt soils (Table 4) are also indicative of the occurrence of oxidative scission of long chain homologues in the soils affected by fire (González-Pérez et al., 2008).

In addition, the relative abundance of isoprenoids was conspicuously reduced in the burnt samples. Isoprenoids are hydrocarbons derived from the degradation of the phytyl-side chain of chlorophyll (Brooks et al., 1969). The greater abundance of pristane than phytane in the burnt than unburnt samples suggested a larger oxic diagenesis for burnt soils.

Namely, pristane/phytane ratios can be used as a measure of sediment oxicity (Didyk et al., 1978; Gagosian et al., 1980; De la Rosa et al., 2009), our result indicates that it can be also a useful marker for detecting fire events in soils.

Conclusions

The presence and relative abundances of homologous compound series as determined by analytical pyrolysis provided meaningful information on effects of wildfire on the quality of soil organic matter (SOM) for both maritime pine and eucalypt plantations.

Thermal desorption step (300 ºC) revealed that the wildfire produced SOM modifications that mainly consisted of a reduction in the more thermally-labile molecular structures. Pyrolysis at 500 ºC, on the other hand, revealed an accumulation of low-molecular weight homologues of certain chemical series, suggesting the occurrence of thermal breakdown and cracking of long-chain SOM components exerted by fire. Shifts in the relative abundance of several markers such are polysaccharide-, lignin-derived compounds and triterpenes were consistent with a wildfire of low severity, as also suggested by field observations (scorching of the tree canopies and the predominance of black ashes).

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2.2. Wildfire-induced alterations of topsoil organic matter and their recovery in Mediterranean eucalypt stands detected with biogeochemical markers (Article II)

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Abstract

This study addressed fire-induced changes in topsoil organic matter (SOM) from an eucalypt plantation in Portugal over two years by using three complementary biogeochemical techniques: elemental analysis, analysis of biomarkers from the total extractable lipids (TLE) and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Direct wildfire effects included a marked decrease in soil total organic carbon (TOC) and total nitrogen (TN) content. However, both contents recovered during the two years. Fire also reduced substantially the TLE, producing noticeable changes in its composition. These included the thermal breakdown and cracking of n-alkyl compounds. Ratios of short-to-long *n*-alkanes and *n*-fatty acid methyl esters (FAMEs) increased and typical carbon number predominance indexes for *n*-alkanes (odd-to-even) and *n*-FAMEs (even-to-odd) were altered. Furthermore, the relative abundances of certain markers which are plant-species specific were modified, especially by decreasing terpenoids such as epiglobulol, ledol and globulol which are characteristic of *Eucalyptus globulus*. Other differences observed in the burnt soil were the appearance of levoglucosan, a typical marker for the thermal alteration of polysaccharides, larger relative abundances of lignin-derived compounds (vanillin and methoxyphenols) and the presence of N-heteroaromatic structures, that suggested the accumulation of 'black nitrogen'. The ^{13}C NMR spectra indicated that the wildfire produced a considerable increase in the aromaticity and aromatic condensation of the topsoil SOM. This was reflected in a broadening of the signal of aromatic compounds at the expense of O-alkyl and alkyl-C compounds. The continuation of these differences in SOM quality during the

two year-study, suggested a slow recovery of soil properties, possibly influenced by a limited recovery of the vegetation after the fire combined with the fire-enhanced losses of soil.

Introduction

Wildfires drive the behaviour of many biomes across the world, including in the Mediterranean where they are considered to be one of the main causes of soil degradation (Shakesby, 2011). In Portugal, as in the European Mediterranean region as a whole, the frequency and extent of wildfires increased dramatically from the 1960s, because of warmer and drier climate conditions and, in particular, socio-economic changes such as land abandonment and the widespread planting fire-prone tree species (Shakesby, 2011). Continuation of this intensified fire regime are expected to cause important changes in soil organic matter (SOM) quantity and quality and to affect soil carbon sequestration as well as related soil ecosystem services (González-Pérez et al., 2004).

Although many Portuguese ecosystems are fire-prone, the response of their SOM to fire is still poorly known. In general, the effects of fire on SOM content vary greatly, depending strongly on fire intensity and fuel load characteristics. As soil is a good heat insulator (González-Pérez et al., 2004), and effects will decrease markedly with soil depth. Even at 2−3 cm depth, wildfires rarely produce increases of soil temperature that are large enough to result in serious changes in soil and SOM properties, so that recent studies have increasingly focused on the topsoil for assessing heating-induced changes (Badía-Villas et al., 2014).

Fire effects on SOM tend to be very complex. Changes in topsoil SOM content range from total destruction by very intense fires to increases of up to 30% (De la Rosa et al., 2013). Alterations in SOM quality typically consist of an increase in aromatic C through charring at the expense of a decrease in thermo-labile O-alkyl C, and of a conversion of peptide N into heterocyclic structures (Knicker et al., 2006). While such marked re-arrangements of C forms by heating can include specific aspects such as, for example, the formation of new water-repellent substances(Atanassova & Doerr, 2011; de Blas et al., 2013), it tends to affect soil health and quality as a whole, including biodiversity and biological activity (González-Pérez et al., 2004). In many cases, however, fire effects on SOM are thought to be reversible, reverting naturally to (almost) pre-fire conditions (González-Pérez et al., 2008).

The recovery of SOM from fire effects continues to be an important research gap. This includes the identification of biogeochemical compounds that are suitable as indicators of the recovery of SOM quality. The soil lipid composition is of special interest, as it is known to be a relatively labile soil OM fraction that is prone to rapid and significant alterations by heating (González-Pérez et al., 2008). The soil free lipid fraction corresponds to a diverse group of hydrophobic substances ranging from simple compounds such as fatty acids, to more complex substances such as sterols, terpenes, polycyclic aromatic hydrocarbons, waxes and resins. These substances play an important role in the incorporation of plant material into SOM, and contain several diagnostic markers for determination of source apportionment and turnover rate (Knicker et al., 2013). González-Pérez et al. (2008) also suggested their potential suitability as proxies of the evolution of SOM quality with time-since-fire. Alkyl lipid compounds such as alkanes, fatty acids and alcohols seem to be suitable indicators of high-intensity fires in particular, as high temperatures seem to cause a reduction on their chain length (Tinoco et al., 2006; Knicker et al., 2013). Aliphatic and aromatic hydrocarbons have been suggested by Wiesenberg et al. (2009) as offering specific fingerprint indicators of fire intensity/severity and burnt vegetation type, including those in fire-affected fossil soils.

Several studies have suggested that certain compounds of the soil lipid fraction respond in a specific manner to heating. Examples of such compounds are terpenoids (Almendros et al., 1988), benzenecarboxylic acids (González-Pérez et al., 2004), and various compounds of the alkyl domain (*n*-alkane/alkene pairs and fatty acids) (González-Pérez et al., 2008). In using such lipids as biomarkers, however, due consideration needs to be given to possible alterations when these compounds enter into the soil, either by microbial degradation processes or by re-synthesis of microbial biomass. Furthermore, other sources of such compounds need to be considered as well, in particular as lipids can also derive from decaying root residues and root exudations (Knicker et al., 2013).

Lignin derivatives are widely held to be an important group of biomarkers of fire effects. Lignin is the most abundant polymeric aromatic organic substance in plants and can account for 20−40 % of wood biomass (Sharma et al., 2004). Knicker et al. (2008) demonstrated that the lignin backbone can survive charring of plant material. However,

it is not yet well understood to which extent the input of such charred lignin residues affects the lignin pool in fire-affected soils. Lignin degradation is a complex process, which includes competitive and/or consecutive reaction steps due to its hindered structure. Lignin decomposes over a broad range of temperatures, because the various oxygen-based functional groups have different thermal stabilities with scissions occurring at different temperatures. While the lignin structure starts to decompose at relatively low temperatures of 150–275° C, the aliphatic side chains start to split off from the aromatic ring at around 300° C and the carbon-carbon bonds between lignin structural units start to cleavage at 370–400° C. At 500–700° C, complete rearrangement of the lignin backbone takes place, resulting in 30–50 wt% char and the release of volatile products (CO, CO_2, CH_4, H_2) (Sharma et al., 2004).

Our study aims to contribute to a better knowledge of wildfire effects on topsoil SOM and, in particular, on the post-fire recovery of its lipid composition. To the best of our knowledge, this topic has never been addressed in Portugal, in spite of the present-day intensified fire regime and its widespread planting with fire-prone tree species. The study's specific objectives were to (i) assess the direct effects of fire-induced heating on the content and composition of total extractable lipid as well as several ancillary soil properties, by comparing two nearby eucalypt plantations burnt in 2010 with long-term unburnt plantations and (ii) evaluate the recovery of the topsoil from these direct effects by determining the changes in total extractable lipids at both study sites at six-monthly intervals during the first two years after the fire. To this end, we used the three complementary techniques, of elemental analysis, analysis of biomarkers from the total extractable lipids (TLE), and solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy.

Materials and Methods

Study area, study sites and soil sampling

The study area was located near the hamlet of Ermida, in the Sever do Vouga municipality, in the Aveiro district of north-central Portugal (40° 08′ 46″ N; 7° 59′ 35″ W; 500 m above sea level (Figure 10). Between the 26th and 28th of July 2010, a wildfire burnt approximately 300 ha of forested lands, predominantly consisting of eucalypt plantations (*Eucalyptus globulus* Labill.). The severity *sensu* Keeley (2009) appeared to be moderate, as assessed by tree crown scorching, litter consumption and ash colour as indicators and in line with prior studies in the region, such as Prats et al. (2014).

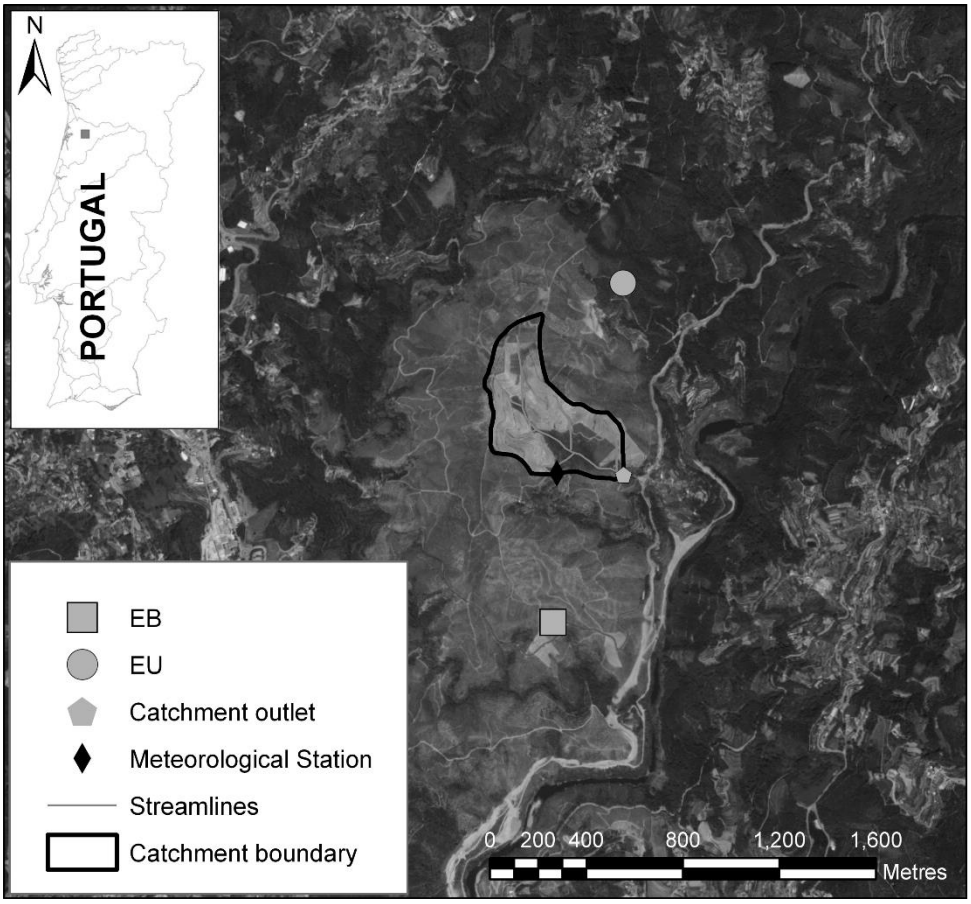

Figure 10 - Location of the Ermida study area and the two study sites, burnt (EB) and long-unburnt (EU) eucalypt plantations.

The climate of the study area is classified as humid meso-thermal (Csb in the Köppen classification) having moderately dry but extended summers. The long-term annual temperature was 14.9°C, at the nearest weather station (Castelo Burgães: 40° 51′16″N;

8°22'55"W; 306 m above sea level during 1990–2010) and long-term mean annual rainfall was 1609 mm, at the nearest rainfall station ("Ribeiradio": 40° 44'39"N; 8°18'05"W; 228 m above sea level during, 1990–2010) (SNIRH, 2011). The study area belongs to the Hespheric Massif, one of the region's major physiographic units, and is composed mainly of pre-Ordovician schists and graywackes, although Hercynian granites do occur at several locations (Ferreira de Brum, 1978).

For the present study, two neighbouring eucalypt plantations were selected (Table 4). One was located within the burnt area, designated EB (40°43'30 "N; 8°20'57"W; 150 m above sea level), while the other was located beside the burnt area, designated EU (40°44'15"N; 8°20'46"W; 253 m above sea level).

Table 4 - Topsoil (0–2 cm) characteristics at the recently burnt and the long-unburnt eucalypt plantation.

	Eucalypt Burnt (EB)	Eucalypt Unburnt (EU)
Slope angle / ° degrees	20	25
Density / g cm^3	1.15	0.88
Water holding capacity / ml g^{-1}	1.2	0.9
Sand / %	67.3	69.2
Clay / %	23.7	22.0
Silt / %	9.0	8.8
Texture	Sandy clay loam	Sandy clay loam

At the base of the two study sites, several soil profiles were sampled and classified. The various profiles corresponded to Umbric Leptosols (IUSS, 2014), comprising shallow A-horizons of 13–20 cm thickness and C- and R-horizons consisting of schists. At both study sites, soil sampling was carried out immediately after the wildfire in August 2010 to study the direct fire effect and then repeated on four occasions at six–monthly intervals until August 2012 to study the post-fire soil recovery.

At each sampling occasion, the topsoil of 0-2 cm depth was collected at five sampling points for each study site. These five sampling points were located at fixed distances along a transect that was laid out from the bottom to the top of the site and which was shifted 1 m across the slope at subsequent sampling occasions. Before sample

collection, litter (EU) and ash (EB) were carefully removed, although a distinct ash layer was no longer recognizable one year after the fire, possibly through incorporation of the charcoal into the topsoil and/or losses by the widespread overland flow in the study area (Prats et al., 2014). Following field collection, the individual soil samples were air-dried at ambient temperature, passed through a sieve with a mesh width of 2 mm, and then stored under dark and cool conditions prior to analysis. The elemental and physical analysis and the extraction of the total lipid fraction were performed with composite samples (one per site and sampling occasion). These composite samples were obtained by mixing sub-samples of the five samples from the individual transects (in equal dry-weight proportions).

Elemental and physical analysis

Total carbon (TC) and total nitrogen (TN) contents were determined in triplicate (average values were considered as being representative) on homogenized composite soil samples with an elemental analyser (Perkin-Elmer 2400 series II; Perkin-Elmer, Waltham, MA, USA). Total organic carbon (TOC) was determined with a total organic carbon analyser (Shimadzu–TOC-5050A, Tokyo, Japan). Soil pH (in H_2O) was measured with a glass electrode in the supernatant of a 1:2.5 weight/volume mixture of soil and water. Particle size distribution was determined with a standard method as described by Guitián &Carballas (1976) and texture was classified according to the USDA criteria (SSS, 1999). Maximum water-holding capacity (WHC) of the samples was measured according to Richards &Weaver (1944).

Extraction of the total extractable lipid fraction and biomarker analysis

The free lipid fractions of dry soil samples were Soxhlet-extracted over six hours with an automatic BUCHI B-811 extractor (Büchi Labortechnik AG, Flawil, Switzerland) with a dichloromethane-methanol (3:1 v:v) mixture (suprasolv Merck). Dry and homogenized soils (5–10 grams) were weighed in Whatman cellulose thimbles, which were pre-cleaned for 30 minutes with fresh solvent and subsequently dried. The extract was slowly dried while the solvent was automatically removed (González-Vila et al., 2003).

The total extractable lipid (TEL) fractions were obtained by filtering, subsequently concentrated to about 2 ml with a rotary evaporator, dried under pure N stream and stored in desiccators. The TEL yields were determined gravimetrically and related to the total carbon contents of the original samples. An aliquot of the TEL was weighed, re-suspended in dichloromethane (0.5 µl) in a 2 ml vial and dried with sodium sulphate.

Biomarker separation and analysis was conducted with a gas chromatography system Agilent 6890 equipped with a mass selective detector Agilent 5973 (Agilent Technologies, Santa Clara, CA, USA). A fused silica capillary column SE–52 (30 m × 0.32 mm i.d., film thickness 0.25 µm) was used as stationary/separation media and the chromatographic conditions involved increasing oven temperatures from 40 to 100° C at a rate of 30° C $minute^{-1}$ and from 100 to 300° C at a rate of 6° C $minute^{-1}$. Helium was used as carrier gas at a flow rate of 1.5 ml $minute^{-1}$. Mass spectra were acquired at 70 eV ionizing energy. Individual compounds were detected by low resolution mass spectrometry and through comparison with published mass spectra libraries (NIST and Wiley). Traces corresponding to selected homologous series of biomarkers families were obtained by single ion monitoring (SIM) of ions characteristic, such as ion at m/z 57 for *n*–alkanes and ion at m/z 74 for *n*-fatty acid methyl esters (FAMEs). The analysis was performed in duplicate. The relative abundance of the compounds was assessed by determining the relative intensities of the traces to the total chromatogram area using the Agilent ChemStation software (Agilent Technologies, Santa Clara, CA, USA). The corresponding standards deviations were less than 5%, indicating the good reproducibility of the obtained results.

Solid-state ^{13}C nuclear magnetic resonance spectroscopy

Prior to solid-state NMR analysis, soil samples were treated with 10% (v/v) hydrofluoric acid (HF) to remove paramagnetic material that might interfere with the NMR signals as well as to concentrate the OM in the samples (Knicker et al., 2007). It involved shaking 10 g soil in a polyethylene beaker with 50 ml of HF solution for three hour and removing and discarding the supernatant afterwards. This procedure was then repeated five times to obtain a solid residue, containing the concentrated SOM. This residue was then washed three times with distilled water to remove residual HF and, finally, freeze dried.

The solid-state ^{13}C NMR spectra were obtained with a Bruker Avance 600 MHz Wideboard (Bruker Instruments Inc., Germany) operating at a frequency of 150.93 MHz and using zirconium rotors of 4 mm OD with KEL-F-caps. The cross polarization magic angle spinning (CPMAS) technique was applied during magic-angle spinning of the rotor at 15 kHz. A ramped 1H-pulse was applied during the 1 ms contact time to circumvent Hartmann-Hahn mismatches, and a pulse delay of 300 ms was employed. Between 40 000 and 50 000 scans were accumulated, depending on the ^{13}C content of the samples, and line broadenings between 50 and 100 Hz were applied. The ^{13}C chemical shifts were calibrated relative to tetramethylsilane (0 ppm) with glycine (COOH at 176.08 ppm). The spectra were quantified by sub-dividing them into the following different chemical shift regions as described in Knicker (2011): alkyl C (0–45 ppm); N-alkyl/methoxyl C (45–60 ppm); O-alkyl C (60–110 ppm); aromatic C and phenol C (110–160 ppm); carboxyl/amide C and carbonyl C (160–245 ppm). The relative ^{13}C intensity distribution was determined by integrating signal intensity over the above-mentioned chemical shift regions using a modified integration routine as supplied with the instrument's software. At the spinning speed of 15 kHz that we used, the chemical shift anisotropy cannot be completely averaged and causes the appearance of spinning side bands at both sides of the parent material at a distance of the spinning speed (in the present case, between 220 and 275 ppm as well as between 45 and 0 ppm). Therefore, the intensity distribution of spinning side bands were corrected following Knicker et al. (2005).

Results and discussion

Total organic carbon, total nitrogen contents and pH

Immediately after the fire, the TOC concentration was reduced by at least 15% at the burnt site (EB) in comparison with the un-burnt site (EU) (Table 5). The wildfire also seemed to have a pronounced direct effect on topsoil nitrogen, increasing the TN concentration by roughly 75%. As a consequence, the TOC:TN ratio at the EB site one month after the wildfire was less than half that at the EU site. These results were in agreement with those reported by other studies fire-affected Mediterranean soils (Almendros et al., 1988; De la Rosa et al., 2008; Badía-Villas et al., 2014). During the subsequent 24 months after fire, TOC content at the EB site remained consistently below that at the EU site. Differences ranged between 15 and 30% but lacked an obvious seasonal pattern.

Table 5 - Average total organic carbon (TOC), total nitrogen (TN), ratio TOC:TN, pH, and total lipids extract (TEL) of the topsoil (0-2 cm) at the burnt site (EB) and the long-unburnt (EU) eucalypt plantation at 1, 6, 12, 18 and 24 months after a wildfire.

Time after fire (Months)	Code	TOC / g kg^{-1})	TN / g kg^{-1}	TOC:TN	pH (in H_20)	TEL / g kg^{-1} TOC
1	EB1	154	13.5	11	6.0	70.0
	EU1	184	7.8	24	3.1	307.8
6	EB6	96	3.6	27	4.1	80.8
	EU6	127	2.2	58	3.2	308.7
12	EB12	97	4.6	21	4.2	110.3
	EU12	115	2.4	48	3.4	218.2
18	EB18	107	4.2	26	4.3	80.6
	EU18	130	4.5	29	3.4	243.5
24	EB24	84	2.7	31	4.0	108.3
	EU24	118	3.9	30	3.5	199.6

The TOC concentrations were substantially larger immediately after the fire than in the following months at both sites. Specifically, TOC concentrations between months 6 and 24 were 63–71% less at the EU site and 55–69% less at the EB site than during the first month after fire. The large initial TOC concentrations at the EU site could be related to drier summer conditions in 2008 than in 2009 and 2010, limiting breakdown of SOM. For the EB site, the present results are in agreement with Campo et al. (2008), who

attributed a reduction of SOM content in the topsoil (0–5 cm) one year after a fire to slow vegetation recovery and post-fire soil losses. Prats et al. (2014) reported slow vegetation recovery and marked runoff and erosion in other eucalypt plantations in the burnt Ermida area.

Soil pH was markedly higher at the EB site than at the EU site, immediately after fire (6.0 and 3.1, respectively). This agrees with the well-recorded 'liming' effect caused by the fire, through combustion of organic matter (litter, vegetation) and/or the incorporation of bases (hydroxides and carbonates) with the ash (Ulery et al., 1993). During the following months, the pH at the EB site decreased to values of 4.0–4.3 but remained higher than observed in the EU site (3.2–3.5).

Total extractable lipids: content and overall composition

The amount of total extractable lipids (TEL) normalized to TOC sharply decreased after fire and were more than 75% less at the EB than EU site (Table 5). A decrease in the TEL content was previously observed by Wiesenberg et al. (2009) when charring biomass, as well as by Atanassova & Doerr (2011) in soil samples from Australian eucalypt forest heated in a muffle furnace. In contrast, Almendros et al. (1988) found a six-fold increase in extractable lipids in recently burnt than in control soils under *Pinus pinea*.

The present difference in TEL content during the first month after the fire could be attributed to the drastic decrease in above-ground plant biomass, as is typical after fires of moderate to high severity. In contrast, increases in the TEL contents could occur after fires of low intensity through incorporation of decayed plants and partially charred OM into the soil (Tinoco et al., 2006). In the present study, relative differences in TEL contents between the two sites appeared to decrease with time after the fire. After two years the TEL concentration at the EB site was 46% less than that at the EU site (108.3 and 199.6 g kg^{-1}, respectively).

The distributions, the compounds detected and their relative contributions to the TEL fraction, for the two study sites on the five different sampling occasions, are shown in Figure 11 and Table 6.

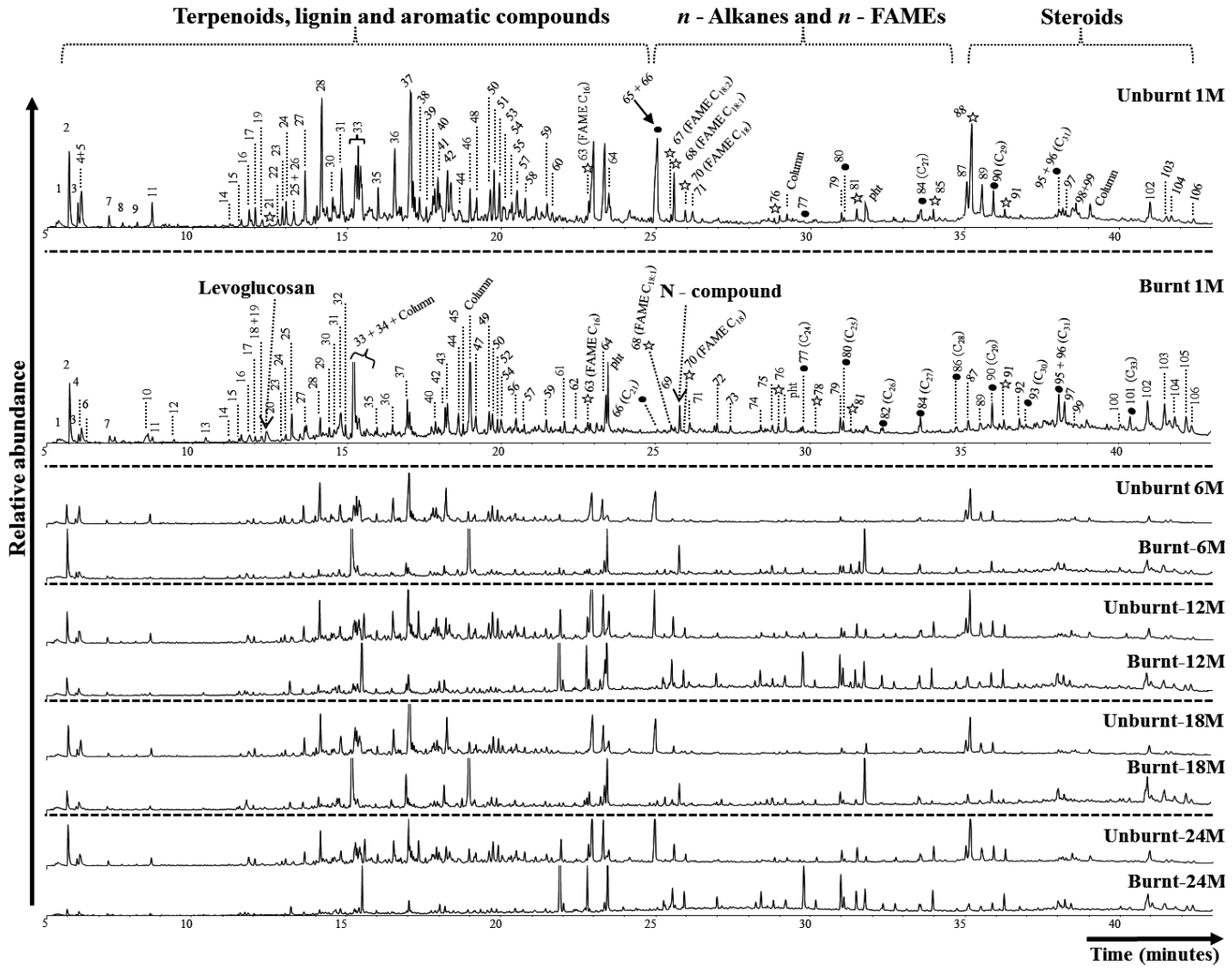

Figure 11 - GC-MS total ion chromatograms (TIC) of burnt (EB) and long-unburnt (EU) Mediterranean topsoils of eucalypt plantations at 1, 6, 12, 18 and 24 months after the wildfire.● *n*-alkanes, ☆ *n*-fatty acids+FAMEs. See Table 3 for the identification of the peaks labels.

Table 6 - Compounds of the topsoil lipid fraction and their relative abundances at the recently burnt and long-unburnt eucalypt plantation at 1, 6, 12, 18 and 24 months after a wildfire, detected using GC-MS and calculated as percentages of the total chromatographic area, respectively.

Peak label	Compound	Molecular formula	MW	Compound family	Retention time/minutes	Burnt (EB)					Unburnt (EU)				
						1M	6M	12M	18M	24M	1M	6M	12M	18M	24M
1	α-Pinene	$C_{10}H_{16}$	136	Terpene	5.64	1.6	1.7	1.2	2.1	1.2	1.0	0.9	1.3	0.8	1.3
2	α-Phellandrene	$C_{10}H_{16}$	136	Terpene	5.98	5.7	7.9	2.9	4.3	1.4	4.3	2.7	3.1	4.2	3.6
3	Benzene, x,x,x,x- tetramethyl	$C_{10}H_{14}$	134	Aromatic	6.27	0.7	1.6	0.4	0.6	0.2	1.1	0.6	0.5	0.8	0.8
4	β-phellandrene	$C_{10}H_{16}$	136	Terpenoid	6.36	1.6	2.3	1.3	1.9	1.0	0.9	0.6	0.8	1.4	0.9
5	1,8-Eucalyptol	$C_{10}H_{18}O$	154	Terpenoid	6.39	nd	nd	nd	nd	nd	1.5	2.6	1.0	1.9	1.5
6	Benzenemethanamine, N,N-dimethyl-	$C_9H_{13}N$	135	N-compound	6.52	0.2	0.6	nd	0.4	nd	nd	nd	nd	nd	nd
7	Benzene, pentamethyl	$C_{11}H_{16}$	148	Aromatic	7.23	0.7	0.9	0.6	0.9	0.5	0.6	0.6	0.7	0.5	0.7
8	Unknown Terpenoid (m/z 154,121,11,93,81)	$C_{10}H_{18}O$	154	Terpenoid	7.72	nd	nd	nd	nd	nd	0.2	0.1	0.2	0.1	0.2
9	Trans-Pinocarveol	$C_{10}H_{16}O$	152	Terpenoid	8.19	nd	nd	nd	nd	nd	0.2	0.2	0.2	0.2	0.2
10	Benzene carboxlic acid	$C_7H_6O_2$	122	Aromatic	8.52	2.1	0.5	0.4	0.8	0.5	nd	nd	nd	nd	nd
11	Borneol	$C_{10}H_{18}O$	154	Terpenoid	8.67	0.6	0.5	0.5	0.5	0.2	1.3	1.2	1.1	1.0	0.9
12	Levoglucosenone	$C_6H_8O_4$	144	Others	9.37	0.6	0.03	0.02	nd	nd	nd	nd	nd	nd	nd
13	Nonanoic acid	$C_9H_{18}O_2$	158	FAMEs+ FA	10.40	0.5	0.6	0.5	0.4	0.2	0.02	nd	0.1	0.1	0.1
14	Thymol	$C_{10}H_{14}0$	150	Terpenoid	11.16	0.2	0.4	0.2	0.2	nd	0.2	0.1	0.1	0.2	0.1
15	2-Methoxy-x-vinylphenol	$C_9H_{10}O_2$	150	Lignin	11.48	0.3	0.3	0.2	0.4	0.2	0.1	0.05	nd	0.1	nd
16	Alkly benzene	C_xH_y	200	Aromatic	11.82	1.2	1.7	0.7	1.0	0.6	1.1	0.4	1.0	1.0	1.2
17	Longifolene	$C_{15}H_{24}$	204	Terpenoid	12.01	0.4	0.7	0.4	0.7	0.3	0.8	0.6	0.6	0.9	0.7
18	Phenol, 2,6-dimethoxy (Syringol)	$C_{10}H_{12}O_4$	196	Lignin	12.21	0.5	0.4	0.4	0.4	0.2	nd	nd	nd	nd	nd
19	Dehydroaromadendrene	$C_{15}H_{22}$	202	Terpenoid	12.24	0.2	nd	nd	nd	nd	0.5	0.5	0.3	0.4	0.2
20	Levoglucosan	$C_6H_{10}O_5$	162	Others	12.38	1.1	0.6	nd	nd	nd	nd	nd	nd	nd	nd
21	Decanoic acid	$C_{10}H_{20}O_2$	172	FAMEs+FA	12.48	nd	nd	0.3	0.6	nd	0.1	0.1	0.1	0.1	0.1
22	Dehydroaromadendrene	$C_{15}H_{22}$	202	Terpenoid	12.75	nd	nd	nd	nd	nd	0.4	0.2	0.3	0.3	0.2
23	β-guaiene	$C_{15}H_{24}$	204	Terpenoid	12.88	0.2	0.3	0.1	0.1	nd	0.7	0.7	0.4	0.3	0.4
24	Unknown Terpenoid (m/z 204, 161, 134, 119,105)	$C_{15}H_{24}$	204	Terpenoid	13.00	0.5	0.4	0.3	0.4	0.3	1.1	1.0	0.7	0.7	0.7
25	Vanillin	$C_8H_8O_3$	152	Lignin	13.20	2.7	1.6	2.1	1.6	2.0	nd	nd	nd	nd	nd
26	Unknown terpenoid (m/z 204, 161, 119,105,93)	$C_{15}H_{24}$	204	Terpenoid	13.26	nd	nd	nd	nd	nd	0.9	0.9	1.0	0.6	0.7
27	α-Gurjunene	$C_{15}H_{24}$	204	Terpenoid	13.62	1.0	1.2	1.3	1.5	1.8	2.9	2.7	1.4	2.0	1.4
28	β-Gurjunene	$C_{15}H_{24}$	204	Terpenoid	14.12	1.4	1.5	1.0	1.9	0.7	5.6	6.0	3.5	5.5	3.6

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Peak label	Compound	Molecular formula	MW	Compound family	Retention time/minutes	Burnt (EB)					Unbunt (EU)				
						1M	6M	12M	18M	24M	1M	6M	12M	18M	24M
29	1H-isoindolene-1,3 (2H) dione	$C_8H_5NO_2$	147	N-compound	14.41	2.1	0.6	0.2	0.5	0.3	nd	nd	nd	nd	nd
30	Aromadendrene	$C_{15}H_{24}$	204	Terpenoid	14.57	1.0	1.6	2.2	1.6	2.0	1.1	2.1	1.5	1.5	1.5
31	4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-	$C_{13}H_{16}O_2$	204	Aromatic	14.79	3.6	2.1	1.3	1.6	1.2	2.8	2.7	1.2	2.5	1.4
32	Phenol, 4-methoxy-2,3,6-trimethyl-	$C_{10}H_{14}O_2$	166	Lignin	14.94	1.0	0.6	0.4	0.6	0.4	nd	nd	nd	nd	nd
33	Isoledene	$C_{15}H_{24}$	204	Terpenoid	15.30	0.8	0.9	0.6	0.9	0.4	6.0	9.8	6.7	6.8	5.5
34	9-ethyl-Anthracene	$C_{16}H_{14}$	206	Aromatic	15.38	2.4	2.8	1.8	1.3	1.3	nd	nd	nd	nd	nd
35	Dehydroaromadendrene	$C_{15}H_{22}$	202	Terpenoid	15.97	0.5	0.5	0.6	0.6	0.6	1.4	1.1	1.1	1.0	1.0
36	Epiglobulol	$C_{15}H_{26}O$	222	Terpenoid	16.48	0.9	0.9	1.1	1.6	1.0	4.1	3.8	2.7	4.0	2.5
37	Ledol	$C_{15}H_{26}O$	222	Terpenoid	16.96	4.0	4.1	3.8	7.5	5.5	10.7	13.8	11.2	15.6	11.5
38	Unknown terpenoid (m/z 204, 189,161, 121,107,93)	$C_{15}H_{24}$	204	Terpenoid	17.33	nd	nd	nd	nd	nd	4.0	nd	2.6	nd	2.3
39	Valerenal	$C_{15}H_{22}O$	218	Terpenoid	17.56	nd	nd	nd	nd	nd	0.5	0.5	0.5	0.5	0.5
40	Spathulenol	$C_{15}H_{24}O$	220	Terpenoid	17.88	0.9	0.9	0.6	1.0	0.6	1.9	2.0	1.2	1.4	0.6
41	Xanthotoxin Furanocoumarins	$C_{12}H_804$	216	Terpenoid	17.94	nd	nd	nd	nd	nd	1.2	1.2	0.9	1.3	1.3
42	Globulol	$C_{15}H_{26}O$	222	Terpenoid	18.19	1.9	2.1	2.7	4.1	3.1	2.8	4.3	2.8	5.1	5.0
43	Syringaldehyde	$C_9H_{10}O_4$	182	Lignin	18.26	2.9	1.1	nd	nd	nd	nd	nd	nd	nd	nd
44	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{18}$	198	Aromatic	18.62	2.5	1.8	0.9	1.0	0.2	0.1	0.1	0.01	0.1	0.1
45	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	198	Aromatic	18.77	0.8	0.9	0.4	0.7	0.2	nd	nd	nd	nd	nd
46	(-)-Caryophyllenol	$C_{15}H_{24}O$	220	Terpenoid	18.98	nd	nd	nd	nd	nd	1.6	1.6	1.5	1.5	1.5
47	Acetosyringone	$C_{10}H_{12}O_4$	196	Lignin	19.16	2.2	2.0	1.2	1.8	0.8	nd	nd	nd	nd	nd
48	Unknown terpenoid (m/z 220,202,187,159,121,107,93)	$C_{15}H_{24}O$	220	Terpenoid	19.20	nd	nd	nd	nd	nd	1.3	1.2	1.4	1.4	1.3
49	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	$C_{10}H_{12}O_4$	196	Lignin	19.59	1.3	0.8	0.7	1.2	0.6	nd	nd	nd	nd	nd
50	Unknown terpenoid (m/z 218,178, 139,121, 107,93)	$C_xH_yO_z$	218	Terpenoid	19.64	0.7	1.1	0.9	1.7	1.0	1.9	1.5	1.8	1.3	1.4
51	Unknown terpenoid (m/z 236, 218,178, 122, 107,95,82)	$C_xH_yO_z$	236	Terpenoid	19.76	nd	nd	nd	nd	nd	2.2	1.9	3.0	2.3	2.5
52	4,7-Epoxy-5(11)-megastigmen-9-ol	$C_{13}H_{22}O_2$	210	Terpenoid	19.86	1.0	0.9	0.7	1.5	0.7	nd	nd	nd	nd	nd
53	Methyleugenol	$C_{11}H_{14}O_2$	178	Lignin	19.94	nd	nd	nd	nd	nd	2.0	1.5	1.9	1.6	1.7
54	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	FAMEs+ FA	19.98	1.6	0.4	0.9	0.6	nd	1.7	0.2	0.6	1.1	0.6
55	Unknown terpenoid (m/z 218,203,175, 136,121,105,91)	$C_{15}H_{22}O_2$	218	Terpenoid	20.34	nd	nd	nd	nd	nd	1.5	0.6	0.6	0.6	0.6
56	1-Phenanthrenol, 1,2,3,4,4a,9,10,10a-octahydro-4a-	$C_{15}H_{20}O$	216	Terpenoid	20.46	0.9	1.7	0.9	1.4	0.9	nd	nd	nd	nd	nd
57	Unknown terpenoid (m/z 234,216,198,183,105,91)	$C_xH_yO_z$	234	Terpenoid	20.51	0.4	nd	nd	nd	nd	1.5	0.7	1.5	1.7	nd
58	3-Hydroxybisabola-1(6),10-dien-2-one	$C_{15}H_{24}O_2$	236	Terpenoid	20.78	nd	nd	nd	nd	nd	1.4	0.5	1.1	0.7	1.1

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Peak label	Compound	Molecular formula	MW	Compound family	Retention time/minutes	Burnt (EB)					Unbunt (EU)				
						1M	6M	12M	18M	24M	1M	6M	12M	18M	24M
59	Phytone	$C_{18}H_{36}O$	268	Terpenoid	21.44	1.3	1.0	0.8	1.2	0.8	0.9	0.9	1.1	0.9	1,0
60	Unknown terpenoid (m/z 234,216,201,173,135,91)	$C_{15}H_{24}O_2$	236	Terpenoid	21.65	nd	nd	nd	nd	nd	nd	0.3	0.6	nd	0.5
61	Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	$C_{16}H_{22}$	214	Aromatic	22.04	1.1	1.5	1.7	0.7	1.7	nd	nd	nd	nd	nd
62	2-Heptadecanone	$C_{17}H_{34}O$	254	Others	22.34	0.5	0.4	0.3	0.5	0.2	nd	nd	nd	nd	0.2
63	FAME C_{16}	$C_{17}H_{34}O_2$	270	FAMEs+ FA	22.78	0.9	1.1	3.9	1.5	4.5	0.7	0.2	1.9	0.3	1.7
64	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	FAMEs+ FA	23.37	1.8	1.7	2.7	2.1	1.8	0.5	0.8	0.9	0.8	0.6
65	Unknown terpenoid (m/z 252,234,224,209,168,107)	$C_xH_yO_z$	252	Terpenoid	25.01	nd	nd	nd	nd	nd	nd	7.7	6.7	6.8	6.7
66	*n*-Henicosane (C_{21} alkane)	$C_{21}H_{44}$	296	Alkane	25.51	0.5	0.7	1.4	0.4	1.5	nd	nd	nd	nd	nd
67	FAME $C_{18:1}$	$C_{19}H_{36}O_2$	296	FAMEs+ FA	25.55	0.2	0.3	3.4	0.9	3.2	1.9	0.6	1.7	0.8	1.6
68	FAME $C_{18:2}$	$C_{19}H_{34}O_2$	294	FAMEs+ FA	25.57	nd	nd	0.2	nd	0.3	0.5	0.2	0.5	0.1	0.6
69	N-methyl-N-benzyldodecanamine	$C_{20}H_{35}N$	289	N-compound	25.79	0.8	0.8	nd	0.6	nd	nd	nd	nd	nd	nd
70	FAME C_{18}	$C_{19}H_{38}O_2$	298	FAMEs+ FA	25.93	0.4	0.6	2.2	0.6	2.9	0.6	0.4	0.9	0.4	0.7
71	Oleic acid	$C_{18}H_{34}O_2$	282	FAMEs+ FA	26.11	1.1	0.8	0.9	nd	nd	0.6	0.3	nd	0.4	nd
72	*n*-Docosane (C_{22} alkane)	$C_{22}H_{46}$	310	Alkane	26.99	0.7	1.0	1.7	0.6	2.1	nd	nd	nd	nd	0.3
73	Phenanthrene, 1-methyl-7-(1-methylethyl)-	$C_{18}H_{18}$	234	Aromatic	27.42	0.6	0.9	0.5	0.5	0.3	nd	nd	nd	nd	nd
74	*n*-Tricosane (C_{23} alkane)	$C_{23}H_{48}$	324	Alkane	28.40	0.5	1.0	1.8	0.4	2.6	nd	nd	0.5	nd	nd
75	N-Methyl-N-benzyltetradecanamine	$C_{22}H_{39}N$	317	N-compound	28.79	0.5	0.4	nd	0.6	nd	nd	nd	nd	nd	nd
76	FAME C_{20}	$C_{21}H_{42}O_2$	326	FAMEs+ FA	28.82	nd	nd	1.1	nd	1.5	0.3	0.1	0.4	nd	0.5
77	*n*-Tetracosane (C_{24} alkane)	$C_{24}H_{50}$	338	Alkane	29.78	0.8	1.3	1.6	0.9	2.5	nd	nd	0.7	nd	0.2
78	FAME C_{21}	$C_{22}H_{44}O_2$	340	FAMEs+ FA	30.18	0.2	nd	0.8	nd	1.7	nd	nd	0.4	nd	0.5
79	1-Tricosanol	$C_{23}H_{48}O$	340	Others	30.99	1.0	1.9	4.1	2.6	5.7	0.4	0.5	0.8	0.6	1.1
80	*n*-Pentacosane (C_{25} alkane)	$C_{25}H_{52}$	352	Alkane	31.09	0.6	1.6	2.3	0.6	2.2	0.1	0.2	0.5	0.2	0.3
81	FAME C_{22}	$C_{23}H_{46}O_2$	354	FAMEs+ FA	31.49	0.2	0.9	1.4	1.1	1.8	0.5	0.2	1.3	0.3	1.5
82	*n*-Hexacosane (C_{26} alkane)	$C_{26}H_{54}$	366	Alkane	32.35	0.3	1.5	1.6	0.3	1.1	nd	nd	0.4	nd	nd
83	FAME C_{23}	$C_{24}H_{48}O_2$	368	FAMEs+ FA	32.75	nd	nd	0.9	nd	1.5	nd	nd	0.3	nd	0.6
84	*n*-Heptacosane (C_{27} alkane)	$C_{27}H_{56}$	380	Alkane	33.57	1.1	2.1	1.6	1.2	1.1	0.3	0.3	0.6	0.4	0.4
85	FAME C_{24}	$C_{25}H_{50}O_2$	382	FAMEs+ FA	33.96	nd	0.5	2.1	0.7	2.5	0.5	0.1	1.5	0.2	1.6
86	*n*-Octacosane (C_{28} alkane)	$C_{28}H_{58}$	394	Alkane	34.74	0.4	1.4	0.8	0.2	0.4	nd	nd	nd	nd	nd
87	Cholesterol (Cholest-5-en-3b-ol)	$C_{27}H_{46}O$	386	Steroid	35.06	1.6	1.2	0.7	1.2	1.0	2.3	1.9	2.4	2.3	2.3
88	FAME C_{25}	$C_{26}H_{52}O_2$	396	FAMEs+ FA	35.14	nd	nd	2.2	1.9	1.3	5.1	4.0	0.2	2.4	1.2

(continuation)

Peak label	Compound	Molecular formula	MW	Compound family	Retention time/minutes	Burnt (EB)					Unbunt (EU)				
						1M	6M	12M	18M	24M	1M	6M	12M	18M	24M
89	Cholest-4-ene-3,6-dione	$C_{27}H_{42}O_2$	398	Steroid	35.55	1.3	1.1	1.3	1.0	0.6	1.4	0,9	1.3	1.0	1.4
90	*n*-Nonacosane (C_{29} alkane)	$C_{29}H_{60}$	408	Alkane	35.98	1.6	2.3	1.7	2.5	1.2	1.1	1.3	1.6	1.3	1.7
91	FAME C_{26}	$C_{27}H_{54}O_2$	410	FAMEs+ FA	36.27	nd	0.9	1.0	0.9	1.1	0.3	0.2	1.0	0.2	1.1
92	Campesterol (ergost-5-en-3b-ol))	$C_{28}H_{48}O$	400	Steroid	36.78	1.1	0.9	0.7	0.8	0.9	0.7	0.5	0.7	0.6	0.7
93	*n*-Triacontane (C_{30} alkane)	$C_{30}H_{62}$	422	Alkane	37.00	0.6	0.6	0.4	nd	0.3	nd	nd	nd	nd	nd
94	FAME C_{27}	$C_{28}H_{56}O_2$	424	FAMEs+ FA	37.37	nd	nd	0.8	nd	0.7	nd	nd	nd	nd	nd
95	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394	Steroid	38.04	1.6	2.1	0.6	0.6	0.4	0.3	0.5	0.4	0.3	0.3
96	*n*-Hentriacontane (C_{31} alkane)	$C_{31}H_{64}$	436	Alkane	38.05	3.0	2.1	1.5	2.8	0.4	0.2	0.1	0.1	nd	0.1
97	Stigmastan-3,5-diene	$C_{29}H_{48}$	396	Steroid	38.24	2.0	1.6	1.4	1.2	1.4	0.2	0.2	nd	0.2	0.3
98	FAME C_{28}	$C_{29}H_{58}O_2$	438	FAMEs+ FA	38.44	nd	nd	1.2	nd	1.1	nd	nd	0.6	nd	0.7
99	D,α,-Tocopherol	$C_{29}H_{50}O_2$	430	Steroid	38.59	0,3	0.3	0.3	0.4	0.3	0.5	0.4	nd	0.3	0.3
100	D:A-Friedoolean-6-ene	$C_{30}H_{50}$	410	Terpenoid	40.04	0.7	1.3	1.1	1.4	0.7	nd	nd	nd	nd	nd
101	*n*-Tritriacontane (C_{33} alkane)	$C_{33}H_{68}$	464	Alkane	40.36	1.7	1.2	0.7	1.2	0.9	nd	nd	nd	nd	nd
102	β-Sitosterol (Stigmast-5-en-3b-ol)	$C_{29}H_{50}O$	414	Steroid	40.93	1.3	1.0	0.8	1.4	1.2	2.2	2.0	4.2	2.7	4.0
103	3-Keto-urs-12-ene	$C_{30}H_{48}O$	424	Terpenoid	41.48	4.3	2.1	2.6	5.9	4.6	0.6	0.8	0.6	0.7	1.0
104	Stigmastan-3b-ol	$C_{29}H52O$	416	Steroid	41.67	0.9	1.3	0.9	1.6	1.1	0.1	0.5	0.5	0.5	0.4
105	α-Amyrin	$C_{30}H_{50}O$	426	Terpenoid	42.18	2.2	1.1	1.4	3.4	1.6	nd	nd	0.2	0.1	0.2
106	Stigmasta-3,5-dien-7-one	$C_{29}H_{46}O$	410	Steroid	42.35	1.2	1.7	1.4	1.3	1.7	0.3	0.2	0.3	0.3	0.3

In total, 106 compounds were detected and classified into eight main groups according to their structure and origin. These eight main groups comprised terpenes and terpenoids, steroids, *n*-alkanes, *n*-fatty acid methyl esters (FAMEs), lignin- derived compounds, aromatic compounds, nitrogenated (N) compounds and other non-specific compounds.

An overview of the relative abundances (calculated as percentages of the total chromatographic area) of these eight main groups of compounds is given in Figure 12.

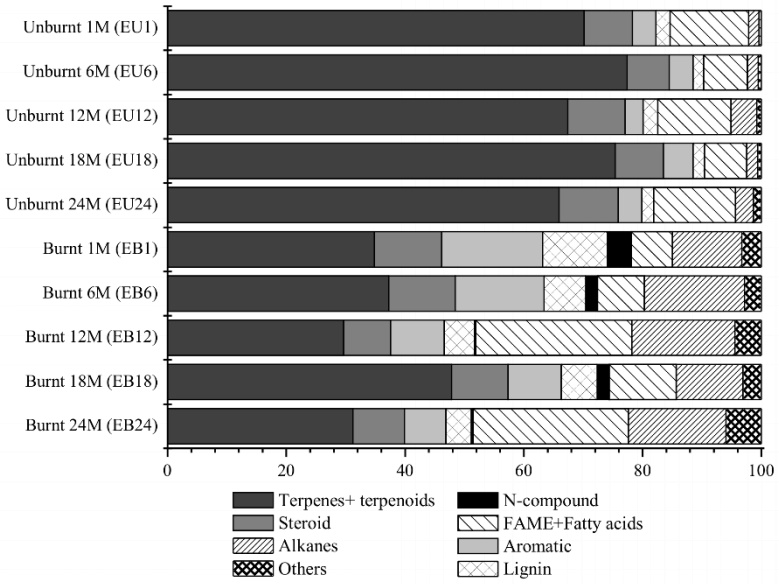

Figure 12 - Cumulative relative abundances calculated as percentages of the total chromatographic area of the major types of compounds identified in soil lipids fraction in burnt (EB) and long-unburnt topsoils (EU) under eucalypt at 1, 6, 12, 18 and 24 months after the wildfire.

Terpenes, terpenoids and steroids were the groups with the largest relative abundance, and were detected in all the samples. These are major biomarkers components of gums and mucilages from angiosperms and, in particular, grasses, shrubs or herbaceous vegetation. Their relative abundance ranged from 38 to 58% of the TEL in the EB samples and from 76 to 84% in the EU samples (Figure 12). Immediately after the fire, their abundance was 50% smaller at the EB site than at the EU site, and this difference was maintained until 24 months after the fire. Aromatic compounds, lignin-derived compounds and N-compounds were present with a larger relative abundance in the TEL of EB site (11–32%) than in the TEL of the EU site (6–8%). The fire seemed to have

produced a strong increase (80%) in the relative abundance of these compounds. Two years later, their relative abundance was still twice as large at the EB site, despite the decrease with elapsed time (post-fire) of their contributions to the TEL fractions. The *n*-alkanes and *n*-FAMEs tended to be more relative abundant in the EB samples (19–43%) than those in EU (9–17%). Among the non-specific compounds, levoglucosan and 1-tricosanol were the most abundant. Their relative abundance was greater at the EB than EU site (3–6 and 0.4–1.3%, respectively, of the TEL). Levoglucosan in particular has been recognized as a marker of vegetation combustion in earlier studies (Kuo et al., 2008).

Terpenes and terpenoids

The terpenes and terpenoids with the greatest relative abundance at both study sites were α-pinene, α- and β- phellandrene, α-and β-gurjunene, isoledene, epiglobulol, ledol, globulol, pytone and 3-keto-urs-12-ene. These terpenes or terpenoids were reported for eucalypt and grassland soils by Otto & Simpson (2005) and Atanassova & Doerr (2010). These compounds had a larger relative abundance at the EU than at the EB site. The EU sample collected immediately after the fire was dominated by the following terpenoids: 1,8- eucalyptol (peak label 5; 1.5 % of the TIC; Table 6); α-and β-gurjunene (peaks label 27 and 28; 2.9 % and 5.6 %, respectively); isoledene (peak label 33; 6.0 %); epiglobulol (peak label 36; 4.1 %); ledol (peak label 35; 10.7 %); and globulol (peak label 42; 2.8 %). These are indicators of the presence of *Eucalyptus globulus* (Song et al., 2009; de Blas et al., 2013) and occurred with a smaller relative abundance in the EB samples (Table 3). In addition, some terpenoids such as 1, 8-eucalyptol, which is a marker of leaves of *Eucalyptus globulus* (Song et al., 2009), were not found in any of the samples collected at the EB site. In contrast, several other terpenoids were only detected in the EB samples: in particular: 4,7-epoxy-5(11)-megastigmen-9-ol (peak label 52; 1.0 %); 1-phenanthrenol, 1,2,3,4,4a,9,10,10a-octahydro-4a-methyl- (peak label 56; 0.9 %); D:A-friedoolean-6-ene (peak label 100; 0.7 %); and α-amyrin (peak label 105; 2.2 %). According to Simoneit (2002), α-amyrin is a marker of angiosperms.

The presence of specific terpene or terpenoid compounds and the relative abundance of others revealed clear differences between the two sites that continued to exist throughout the study period. Overall, the differences suggested that fire produced a

drastic and long-lasting decrease in the relative abundance of compounds derived from plant remains, in agreement with the findings of Otto et al. (2006), Tinoco et al. (2006) and Atanassova & Doerr (2011).

Steroids

The steroids that occurred with the greatest relative abundances in all the samples were cholesterol (peak label 87), cholest-4-ene-3, 6-dione (peak label 89), campesterol (peak label 92) and β-sitosterol (peak label 102). These are common steroids in the waxes of higher plants (phytosterols), which are frequently found in soils and leaf/litter extracts (see Atanassova & Doerr (2011); De la Rosa et al. (2013)). Simoneit (2002) also reported that β-sitosterol and cholesterol are molecular tracers for angiosperm, gymnosperm and gramineae whereas campesterol is a molecular tracer for gramineae. In addition, some other steroids compounds (stigmastan-3,5,22-trien (peak label 95); stigmastan-3,5-diene (peak label 97); stigmastan 3β-ol (peak label 104); and stigmastan-3,5-diene-7-one (peak label 106)) were detected with a greater relative abundance in the EB samples than in the EU samples (5.7% and 1.0%, respectively; one month after fire). These could be derived from the natural product β-sitosterol through thermal oxidation (Otto & Simpson, 2005).

Cholesterol was present at both sites. This is an non-specific steroid and can also be produced by soil microorganisms and soil fauna (Otto & Simpson, 2005). The use of the precursor: altered sterol ratio can be suggested as an indicator of the degree of recovery of soil following fire (*ibid*). This ratio is calculated as the sum of the relative abundances of the peak areas of the precursor sterols (β-sitosterol; cholesterol and campesterol) in the total chromatogram area, divided by the relative abundances of their degradation products (stigmastan 3β-ol; stigmastan-3, 5-diene-7-one; stigmastan-3, 5-diene; stigmastan-3, 5, 22-trien; and cholest-4-ene-3,6-dione). A similar ratio has been used to monitor the degree of degradation in three different soils (*ibid*). The values for this ratio were 4–7 times greater in the EU than EB soils. The observed differences in sterol compounds and sterol ratio between the two sites were maintained during the two

study years (5 times greater in the EU than EB soils), indicating that the soil at the EB site had not fully recovered from the wildfire in 2010.

Aromatic Structures

Fire caused a pronounced increase in the presence of aromatic compounds (17.4% and 5.5% of the TEL in EB and EU samples, respectively, one month after fire). In general, aromatic structures ranged between 7–17% and 4–6% of the TEL compounds for EB and EU soils, respectively. Similar results have been previously reported by Almendros et al. (1988), González-Pérez et al. (2008) and Atanassova & Doerr (2011).

Benzene carboxylic acid (peak label 10); 4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-one (peak label 31); 9-ethyl-anthracene (peak label 34); naphthalene, 1,6-dimethyl-4-(1-methylethyl) (peak label 44); and anthracene,1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl- (peak label 61) were the aromatic compounds with the largest relative abundances in the EB samples. Most of these compounds are usually released during incomplete combustion processes, as occur during wildfires, and indicate the presence of char (Tinoco et al., 2006). The only aromatic compounds detected in the EU samples were 4,4,5,8-tetramethyl-3,4-dihydro-2H-1-benzopyran-2-one and naphthalene,1,6-dimethyl-4-(1-methylethyl), both of which could be also derived from vegetation residues.

n-alkanes

The *n*-alkanes series ranged from C_{17} to C_{33} and revealed bimodal distribution with a maximum at C_{29} for EU samples and at $C_{24,}$ C_{25} and C_{29} for EB samples. In the TEL, *n*-alkanes represent 11–17% of the EB samples and 2–4% of the EU samples. Figure 13 shows changes in the relative abundance of the *n*-alkanes and Table 7 shows the average chain length (ACL), carbon preference indexes (CPI) and short to long ratio of *n*-alkanes.

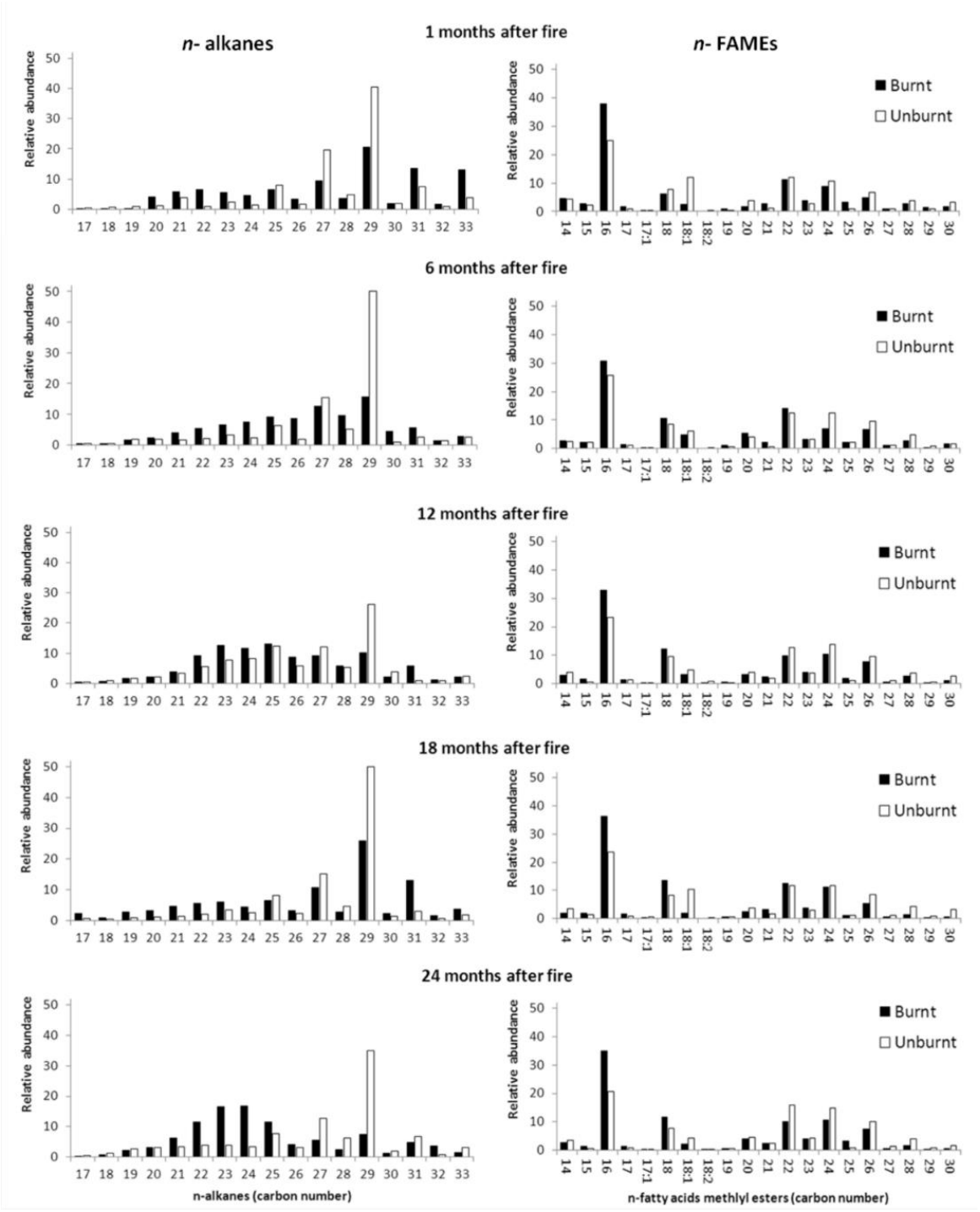

Figure 13 - Relative abundance of *n*-alkanes and *n*-fatty acid methyl esters (FAME's) in burnt (EB) and long-unburnt (EU) topsoils under eucalypt plantation at 1, 6, 12, 18 and 24 months after the wildfire.

Table 7 - Molecular ratios for *n*-alkanes and *n*-fatty acids methyl esters (FAMEs) in the soil lipid fraction at the burnt and long-unburnt eucalypt plantations.

n-Alkanes

	$\sum C_{17}$-C_{25}:$\sum C_{26}$-C_{33} short:long		Diff.	CPI odd:even		Diff.	CPI short		Diff.	CPI long		Diff.	ACL		Diff.
Time (months)	Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt	
1	0.5	0.2	0.3	2.9	6.6	-3.7	1.2	3.9	-2.7	5.4	7.7	-2.3	27.4	27.7	-0.3
6	0.6	0.3	0.3	1.5	5.3	-3.8	1.4	2.0	-0.6	1.5	7.8	-6.3	26.3	27.5	-1.2
12	1.2	0.7	0.5	1.4	2.0	-0.6	1.3	1.5	-0.2	1.5	2.6	-1.1	25.5	26.2	-0.7
18	0.6	0.3	0.3	3.4	5.8	-2.4	1.6	2.4	-0.8	6.4	8.4	-2.0	26.6	27.5	-0.9
24	2.6	0.4	2.2	1.4	3.2	-1.8	1.1	1.6	-0.5	2.4	4.8	-2.4	24.8	26.8	-2.0

n-FAMEs

	$\sum C_{14}$-C_{22}:$\sum C_{23}$-C_{30} short:long		Diff.	CPI even:odd		Diff.	CPI short		Diff.	CPI long		Diff.	ACL		Diff.
Time (months)	Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt		Burnt	Unburnt	
1	2.6	1.9	0.7	4.6	8.8	-4.2	7.5	14.1	-6.6	2.0	4.8	-2.8	19.6	20.6	-1.0
6	2.9	1.6	1.3	6.0	7.3	-1.3	9.3	13.9	-4.6	2.6	3.9	-1.3	19.9	20.8	-0.9
12	2.3	1.6	0.7	6.4	7.8	-1.4	10.1	12.2	-2.1	3.2	4.7	-1.5	19.9	20.9	-1.0
18	2.9	1.6	1.3	6.4	7.8	-1.4	8.8	11.7	-2.9	3.3	4.9	-1.6	19.6	20.9	-1.3
24	2.5	1.5	1.0	6.1	7.2	-1.1	10.7	12.0	-1.3	2.6	4.3	-1.7	19.7	21.2	-1.5

Average chain length = ($\sum Z_n$ x n):$\sum Z_n$), with Z_n as relative amount of *n*-alkanes with *n* carbons.

Carbon preference index of *n*-alkanes = ($\sum C_{17\text{-}33odd}$:$\sum C_{18\text{-}32even}$)

Carbon preference index of short chain *n*-alkanes = ($\sum C_{17\text{-}25odd}$:$\sum C_{18\text{-}24even}$)

Carbon preference index of long chain *n*-alkanes = ($\sum C_{27\text{-}33odd}$:$\sum C_{26\text{-}32even}$)

Diff. = difference between burnt and unburnt site

The distribution of *n*-alkanes showed that immediately after the fire the ACL was slightly reduced in the EB samples (27.4–27.7 one month after fire); however, this difference increased with time (24.8–26.8; two years after fire). Fire also caused a loss of the odd-over-even predominance (CPI decreased for EB samples) and the ratio of short-to-long *n*-alkanes ($\sum C_{17}$-C_{25}:$\sum C_{26}$-C_{33}) increased 2–6 times. This indicates that the fire altered the abundance of native *n*-alkanes by thermal cracking of long chain homologous molecules.

The loss of odd-over-even predominance and an increase in short chain molecules with even numbers of carbon atoms was previously reported for ancient soils subject to biomass burning (Eckmeier & Wiesenberg, 2009), fire affected forest soils (González-Pérez et al., 2008; De la Rosa et al., 2013) and for charred biomass (Wiesenberg et al., 2009; Knicker et al., 2013).

The largest short to long chain ratio ($\sum C_{17}$-C_{25}:$\sum C_{26}$-C_{33}) was reached two years after fire so the differences between EB and EU soils were amplified over this period. The same occurred for the ACL and CPI indexes between EU and EB soils. These changes indicate that the evolution of the molecular composition of the EB soil OM was different to that of the unaffected soil, probably because of fire induced degradation/erosion processes.

n-fatty acid methyl esters (FAMEs)

The series of *n*-fatty acid methyl esters (FAMEs) ranged from C_{14} to C_{30} with a predominance of even-numbered C- chains, and a maximum at C_{16}, C_{22} and C_{24} for all the samples (Figure 4). These compounds represent the 7–26% of the TEL for EB samples and 7–14% for EU samples. Immediately after the fire, the ratio short:long chain increased in the EB sample relative to those of EU soil (2.6 to 1.9; table 7) and these differences were maintained during the two years. The ACL was slightly smaller in EB samples than in the unaffected soils (19.6–19.9 and 20.6–21.2, respectively; Table 4). These results confirm the oxidative scission of long chain homologues in the soils affected by fire as previously described by González-Pérez et al. (2008) and Atanassova & Doerr (2011) without any clear indication of the recovery of the OM at a molecular level to the pre-fire conditions.

It is known that some unsaturated acids ($C_{17:1}$, $C_{18:1}$ and $C_{18:2}$), may be from a bacterial contribution to soil OM (Wiesenberg et al., 2010). Such molecules were detected in both the EB and EU soils. According to Wiesenberg et al. (2010), the ratio of unsaturated to saturated fatty acids [$(C_{18:1} + C_{18:2}):C_{18}$)] may indicate the ongoing degradation of plant biomass towards soil OM. This ratio was calculated as the sum of the relative abundances of the peak areas of the unsaturated acids in the total chromatogram area, divided by the relative abundance of their saturated fatty acids. Figure 14 compares their presence in the EB and EU soils. The decrease in the relative abundance of unsaturated FAMEs in the EB soils could correspond to a diminution in the microbiological population affected by heat. The presence of $C_{18:2}$ one year after the fire in EB samples could result from a seasonal increase in the above-ground biomass of perennial plant species (Elgersma et al., 2003), and was in agreement with the increase of content of TOC observed 12 months after the fire (70 to 110 g/kg).

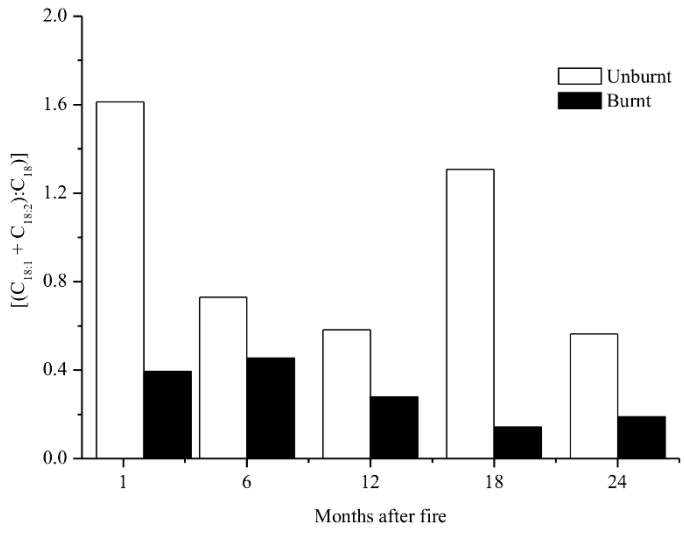

Figure 14 - Ratio of unsaturated to *n*-C_{18} fatty acids in burnt (EB) and long-unburnt (EU) topsoils under eucalypt plantation at 1, 6, 12, 18 and 24 months after the wildfire.

Lignin compounds

Lignin-derived compounds were detected at both sites and its relative abundance ranged from 4 to 11% of the TEL in the EB samples, and was around 2% in the EU samples. These structures are characteristic of plant and wood material (Sharma et al., 2004). The larger relative abundance of lignin compounds in the EB soils could be

explained by their greater thermal stability in comparison with other compound series. Vanillin (peak label 25); syringaldehyde (peak label 43); and acetosyringone (peak label 47) were the lignin compounds that occurred with the largest relative abundances in the EB samples. In contrast, methyleugenol (peak label 53) was the compound with the largest relative abundance in the EU samples, whereas vanillin (peak label 25) was not detected. Vanillin is the primary aromatic alcohol monomer of gymnosperm lignin and also is a marker for pine (Knicker et al., 2013) and eucalypt wood (Nunes et al., 2010), whereas acetosyringone is a marker of angiosperm lignin (Simoneit, 2002). Although with a small relative abundance, several other methoxyphenol compounds (peak labels 18, 32 and 49) were detected in the EB samples. The presence of such compounds could indicate that fire caused a thermal degradation of lignin structures and the incorporation of monomeric lignin compounds to the TEL fraction. These compounds were also detected in the Py-GC/MS chromatogram of the charred plant residues from humic substances (Knicker et al., 2005) and in the burnt soil from a Mediterranean forest (De la Rosa et al., 2008; Knicker et al., 2013).

Nitrogenated (N)-compounds

N-compounds were only detected in the EB site, with a relative abundance of 0.2–3 % of the TEL. For all burnt soil samples the most abundant nitrogenated compounds were 1H-isoindolene-1,3 (2H) dione (peak label 29) and N-methyl-N-benzyldodecanamine (peak label 69). These N-compounds showed a larger relative abundance in the EB sample collected immediately after the fire (3.6% and of the TEL), but decreased in the samples collected 12 months later (0.2% of the TEL). The relative abundance of the iso-indolene compound in the EB samples agreed with the increases in heterocyclic N-compounds reported previously after fire in Mediterranean soils (Knicker et al., 2005; De la Rosa et al., 2008) and are related to the aromatic N-structures (‘black nitrogen’) formed during the heating of soil OM and plant biomass. The greater content of those compounds in the EB than in EU samples, immediately after fire is related to the increase of TN content (13.5 and 7.8 g kg^{-1} for EB and EU samples, respectively, after one month).

The relative abundance of N-compounds and TN content at the EB site was strongly correlated (Spearman rank correlation coefficient of 0.78). In addition, two other N-compounds were detected in the EB samples with a small relative abundance (≤ 0.6%): these were benzenemethanamine, N N-dimethyl-(peak label 6) and N-methyl-N-benzyltetradecanamine (peak label 75) and may be considered as peptide-derived structures.

Other compounds

Levoglucosenone and levoglucosan and (peaks label 12 and 20, respectively) were only detected in the EB samples. These compounds are derived from the thermal decomposition of cellulose and have been proposed as a marker for different types of vegetation combustion, such as softwood, hardwood and grass (Otto et al., 2006; Kuo et al., 2008). The presence of levoglucosan and other anhydrosugars was also detected in the Py-GC/MS chromatogram of burnt soils from a Mediterranean forest (De la Rosa et al., 2013). They represented 1.7% of the TEL immediately after the fire but decreased to 0.6% after six months. These results suggest that the partially-burnt remains of the vegetation deposited on the soil surface were lost by post-fire erosion processes and/or by chemical degradation in the early aftermath of the fire, which agrees with the findings of Knicker et al. (2013). On the other hand, levoglucosan is produced by heating cellulose, a process that involves bond cleavage by transglycosylation, fission and disproportionation reactions (Simoneit, 2002): in addition levoglucosenone can be formed through the dehydratation of levoglucosan. Thus, it can be used as a marker of moderate-high thermal alteration of soil OM.

The alcohol 1-tricosanol was also present with a relative abundance between 1–5.7% and 0.4–1.1% of the TEL for EB and EU soils, respectively. The alcohol homologues >*n*-C_{20} are characteristic of vascular plant wax whereas <*n*-C_{20} may be derived from microbial sources (Simoneit, 2002).

^{13}C NMR spectroscopy

The CP-MAS ^{13}C NMR spectra of the HF-treated soils collected 1, 12 and 24 months after the fire and, their relative intensity distribution are displayed in Figure 15 and Table 8, respectively. The NMR spectra revealed remarkable differences in the chemical composition of SOM in the fire affected and unaffected soils. All spectra of the top 2 cm of EU soils had the typical intensity distribution expected for spectra obtained from soils under forest (Knicker et al., 2005; Otto et al., 2006; De la Rosa et al., 2008) and characterized by two dominant peaks, the signal attributable to carbohydrates in the *O*-alkyl C region between 60 and 110 ppm (34 to 37% of the total ^{13}C-intensity) and the intense signal in the chemical shift region between 0 and 45 ppm, typically assigned to alkyl C (36 to 38 % of the total ^{13}C-intensity) in lipids and amino acids. During the experiment, no major changes in the intensity distribution were observed.

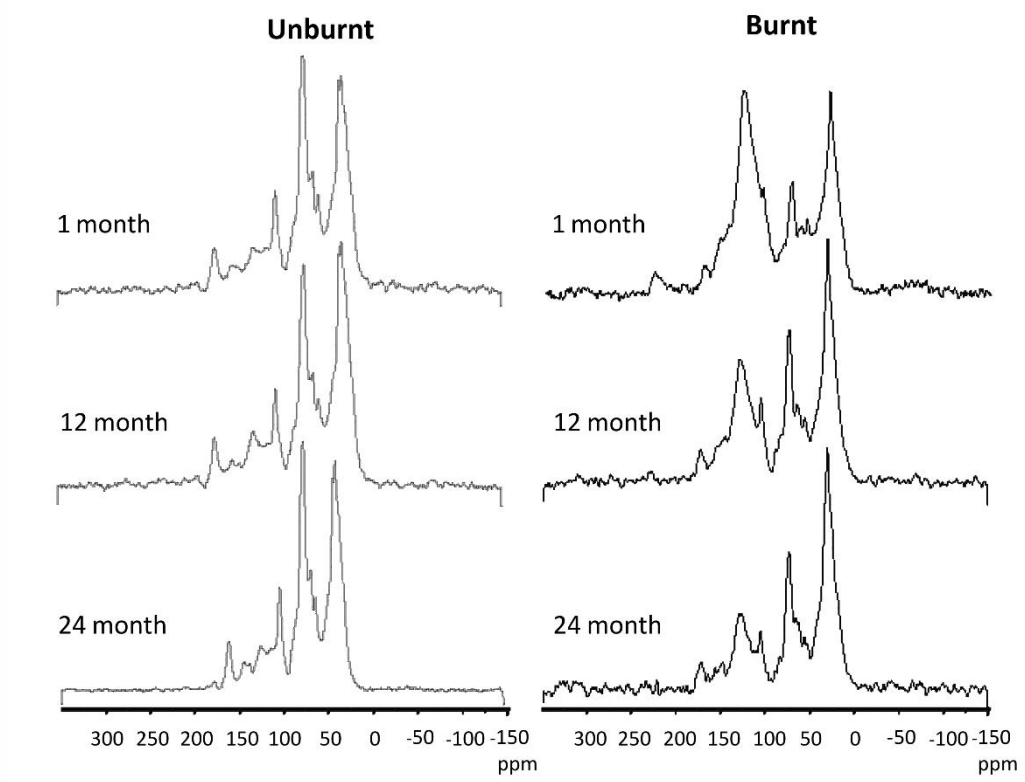

Figure 15 - Solid-state ^{13}C NMR spectra of burnt (EB) and long-unburnt (EU) topsoils under eucalypt at 1, 12 and 24 months after the wildfire.

Table 8 - Relative intensity (% of total intensity) in solid state ^{13}C NMR spectra of burnt (EB) and long-unburnt (EU) soil under eucalypt at 1, 12 and 24 months after the wildfire.

^{13}C NMR data								
Sample	ppm						sum	O-Alkyl/Alkyl
	245–185	185–160	160–110	110–60	60–45	45–0		
EB1	3	3	38	23	7	26	100	0.9
EB12	3	4	28	25	7	34	100	0.7
EB24	2	3	20	26	7	42	100	0.6
EU1	2	4	13	37	9	36	100	1.0
EU12	3	4	13	34	8	38	100	0.9
EU24	1	4	13	37	8	36	100	1.0

Indications for the presence of lignin are given by the signals in the chemical shift region of *O*-substituted aromatic C between 160 and 140 ppm and the signal in the region between 60 and 45 ppm, which is often assigned to methoxyl carbons. However, some response in this region can also be produced by N-alkyl C, associated with peptides and amino sugars. This region contributed 8–9% of the total ^{13}C-intensity in the EU soils. The proportions of aromatic C (160 and 110 ppm) of 13% of the EU samples are typical of soils lacking contributions from charred material (Knicker et al., 2007), and are similar to the 15% reported for fresh plant material by Knicker et al. (2008). Peaks for carboxyl/amide contributed about 4% to the region between 160 and 185 ppm and for carbonyl C 2% to that between 245 and 185 ppm.

The fire apparently resulted in a considerable increase as well as broadening of the signal in the sp^2 C region, at the expense of the *O*-alkyl and alkyl C signal (from 13 to 38%). The intensity of the aryl-C region decreased from 38 to 28 and then to 20 % at one, 12 and 24 months after fire, respectively. Concomitantly, the signal intensity for alkyl C increased from 26 to 42%, probably because of a relative increase in plant residues. However, fatty acids, waxes and resins also contribute to this region (Tinoco et al., 2006).

The signal attributable to carbohydrates in the *O*-alkyl C region (60–110 ppm) was reduced sharply to 23–26% (it ranged 34–37% for the EU samples). It has been widely reported that heating SOM leads to a loss of *O*-alkyl C structures (González-Pérez et al., 2004; Tinoco et al., 2006). The initial vegetation recovery after a wildfire is typically

dominated by herbs and shrubs, and is likely to result in considerable litter production. The present results indicate that post-fire vegetation recovery was rather limited, agreeing with what other post-fire studies in the study region reported (Prats et al., 2014).

The signal corresponding to alkyl C (0–45 ppm) was also reduced in the EB samples, which is in agreement with the breaking of alkyl structures shown from the analysis of the TEL. However, the signal intensity in the region between 60 and 45 ppm, assigned to methoxyl carbons of lignin and hemicelluloses, remained constant with a contribution 7%. The signals at 153 and 145 ppm probably corresponded with C_3 and C_5 of syringyl units and to C_3 and C_4 of guaiacyl, respectively, associated with lignin structures (Knicker *et al.*, 2007). The signal at 105 ppm remained in the spectra of all samples collected after the fire. This region in the signal corresponds to cellulosic structures, although non-protonated aromatic carbons in tannins my also contribute (Mengchang et al., 2008).

Conclusions

The present results indicate that a moderate-severity wildfire produced substantial changes in the quantity and quality of SOM in the topsoil of eucalypt plantations. The wildfire appeared to have resulted in marked decreases in TOC and TEL contents as well as in a clear increase of the TN content. These initial differences between the burnt and the long-unburnt eucalypt plantations diminished during the 24 months following the fire but at a pace that suggested a limited recovery from the direct fire effects. The biomarkers in the TEL fractions indicated that the wildfire modified topsoil SOM by (i) thermal breakdown of the alkyl-chains, increasing the ratios of short-to-long *n*-fatty acids and *n*-alkanes; (ii) increasing and altering the characteristic CPIs; (iii) increasing the relative abundance of aromatic compounds, most probably through the input of charred biomass, as was also suggested by the ^{13}C NMR spectra; (iv) decreasing the relative abundances of specific biomarkers of vegetation and, in particular, *Eucalyptus globulus* such as the terpenoids epiglobulol, ledol and globulol; (v) introducing compounds that

are thermal degradation products from steroids, such as stigmastan-3,5-diene (derived from β-sitosterol); (vi) increasing the relative abundance of lignin compounds, indicating the greater thermal stability of lignin moieties compared with other SOM chemical structures; (vii) introducing levoglucosan, indicating thermal alteration of biomass; and (viii) introducing heteroaromatic N-compounds, pointing towards thermal transformation of SOM.

The limited post-fire recovery of soil over two years reflected the poor growth of vegetation and was observed in other studies in the area and these reported the losses of soil from runoff and erosion. The slow soil recovery is also reflected in the ^{13}C NMR spectra, revealing a weak enrichment in the relative abundance of carbohydrates in the burnt topsoils during the first two years after fire.

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2.3. Molecular characterization of wildfire impacts on organic matter in eroded sediments and topsoil in Mediterranean eucalypt stands (Article III)

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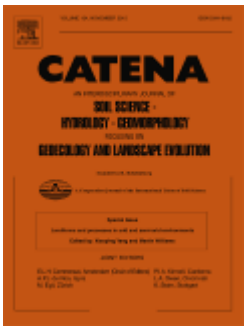

Abstract

Wildfires can increase soil erosion due to the destruction of the vegetation and changes in soil organic matter (SOM) quantity and quality. This fact is especially dramatic in the case of Mediterranean ecosystems. Therefore, the major goal of the present study was to achieve a better understanding of the short−term effects of wildfires on the quantity and quality of organic matter (OM) in the topsoil of burnt Mediterranean eucalypt plantations in north-central Portugal.

Wildfire had a large impact on slope-scale sediment losses, as they were approximately 30 times higher at the burnt site than at the unburnt site over the first 25 months after the fire. The pH and total nitrogen (TN) content of the burnt topsoil showed a noticeable increase immediately after the wildfire, whereas the total organic carbon (TOC) content revealed the opposite behavior. However, the TOC content showed a partial recovery during the subsequent two years after the fire. The sediments eroded after the fire were consistently more acid and richer in TOC and TN than the burnt topsoil, they also revealed less pronounced changes with time-since-fire compared with the burnt topsoil. Analytical pyrolysis analyses indicated that the fire resulted in an enrichment of aromatic compounds, nitrogen (N) constituents, lignin−derived compounds and polysaccharides. The latter is best explained with the input of partially charred OM, ash and decaying parts of plants killed by the fire. The thermal transformation of SOM caused the thermal breakdown and cracking of *n*-alkanes compounds, as was revealed by the increase of the ratio of short-to-long chains and the alteration of the typical odd-over-even carbon predominance indexes. The relative abundance of specific biomarkers for vegetation and, in particular for *Eucalyptus globulus*, such as terpenoids eucalyptol and globulol decreased drastically or even disappeared from the pyrolysates of the burnt

topsoil. These fire-induced changes in the quality of topsoil OM persisted during the whole post-fire period of 25 months. In general, the Py–GC/MS results pointed out that during the first two years after the fire the recovery of the topsoil OM quality was practically negligible.

Introduction

In Portugal, like in other Mediterranean regions in southern Europe, wildfire frequency and extent have increased dramatically during the last decades (Marques et al., 2011; Shakesby, 2011; Caon et al., 2014) and a substantial reduction of the fire frequency is not expected for the next decades (Pereira et al., 2006). This intensification of the fire regime can be attributed to a combination of socio-economic changes, in particular land abandonment and afforestation with fire-prone tree species, such as pine and eucalypt and an increase in meteorological conditions propitious to wildfires (Pereira et al., 2006; Llovet et al., 2009).

As discussed by Shakesby (2011), wildfires can have important effects on soil geomorphologic and hydrological processes. The commonly observed increases in runoff and soil erosion in recently burnt areas are typically attributed to the removal of the protective soil cover by vegetation and litter, in combination with heating-induced changes in topsoil properties such as infiltration capacity, aggregate stability and soil water repellency (Shakesby and Doerr, 2006; Varela et al., 2010; Granged et al., 2011; Badía-Villas et al., 2014a; Prats et al., 2014). The combined direct and indirect effects of fires can have important short−to long−term implications for key aspects of soil quality such as the quantity and quality of soil organic matter (SOM), nutrient stocks and pollutants (e.g polycyclic aromatic compounds), as well as for aquatic habitats receiving sediment− and ash−loaded runoff from upstream burnt areas (González-Pérez et al., 2004; Smith et al., 2011; Campos et al., 2012).

The observed effects of fire on SOM content are highly variable, depending markedly on fire severity, and may range from (almost) complete destruction to increases of up to 30 %, especially in topsoil layers as a result of the incorporation of ash and charred material (De la Rosa et al., 2013). The effects of fire on SOM quality continue an important research gap, also because recent advances and, in particular, the use of pyrolysis gas chromatography/mass spectrometry (Py−GC/MS), also called analytical pyrolysis, have shown that these effects can be very complex (González-Pérez et al., 2004; De la Rosa et al., 2008b; De la Rosa et al., 2012; Aznar et al., 2013; Badía-Villas et al., 2014a). Analytical pyrolysis provides information concerning the structure of organic molecules, including N species, which cannot be released by hydrolysis. This technique involves

thermolytic fractionation of macromolecules into small fragments that are analyzed by gas chromatography –mass spectrometry (GC–MS). Nonetheless, some fire effects on SOM quality are now well-established such as, the increase of aromatic C at the expense of thermo−labile O−alkyl C due to charring as well as the conversion of peptide N into heterocyclic structures (Knicker et al., 2006; De la Rosa et al., 2008b; González-Pérez et al., 2008). Overall, wildfires can lead to a considerable rearrangement of the C forms in SOM and produce a variety of new substances such as water-repellent compounds and a range of pollutants including polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzofurans and polychlorinated biphenyls, which may have important repercussions for soil health and quality (Arias et al., 2005; González-Pérez et al., 2008; González-Pérez et al., 2014). Among these pollutants, especially PAHs have been identified as an important risk for downstream aquatic habitats (Gabos et al., 2001; Campos et al., 2012).

The present study aimed to determine the short-term effects of wildfires on SOM quality, in particular by comparing burnt and unburnt topsoil of Mediterranean eucalyptus plantations on four occasions, starting immediately after the first post-fire rain till two years later. In addition, this study also aimed to advance the knowledge of the molecular composition of the OM that is being exported by post-fire overland flow following a wildfire. Investigation of the OM composition of transported sediments is of crucial significance for a sophisticated evaluation of wildfire effects on terrestrial ecosystems as well as the aquifer and downstream waters. To do so, this study heavily relies on Py−GC/MS.

Materials and Methods

Study area and sites descriptions

This study was carried out in two eucalypt plantations (*Eucalyptus globulus* Labill.) located near the Ermida hamlet (40° 08' 46" N; 7° 59' 35" W; 500 m a.s.l.) of the Sever do Vouga municipality, Aveiro District, north-central Portugal (Fig. 16). One of the eucalypt plantations (EB: Eucalypt Burnt) was burnt by a wildfire with moderate severity (Faria et al., 2015; Machado et al., 2015) occurring between the 26th and 28th of July 2010.

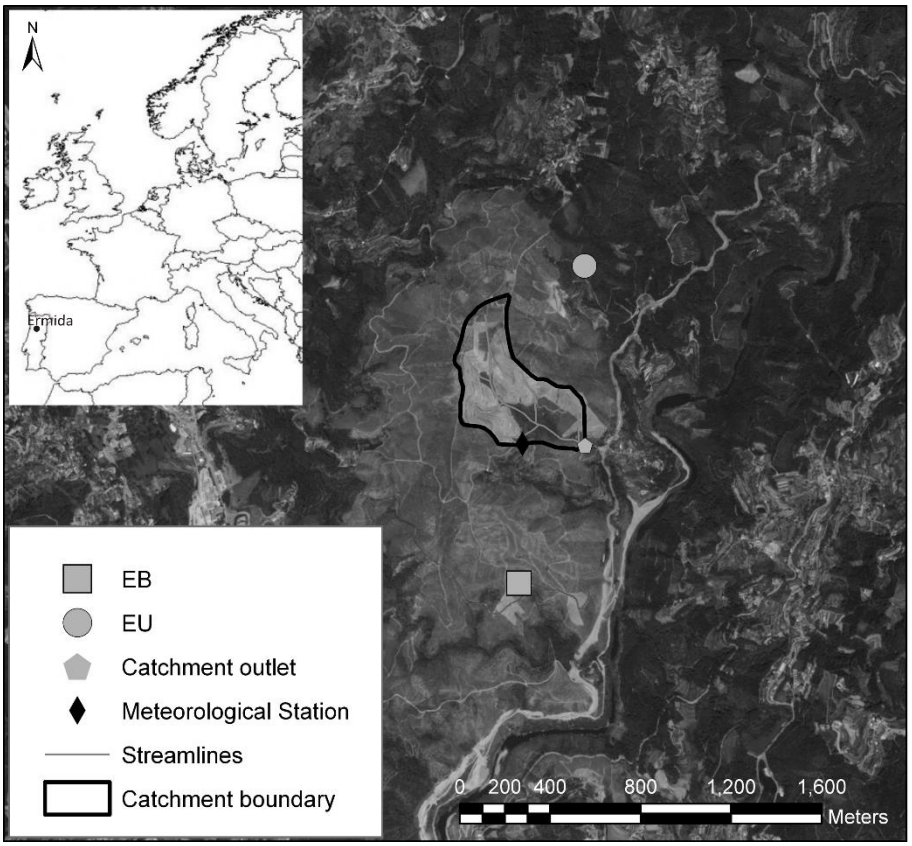

Figure 16 - Location of the Ermida study area and the two study sites, burnt (EB) and unburnt (EU) eucalypt plantations.

Approximately 300 ha of forested lands were consumed. The other eucalypt plantation (EU: Eucalyptus unburnt) was located just outside the border of the burnt area, with physico−environmental settings comparable to those of the EB site.

The study area has a humid meso-thermal Mediterranean climate (Csb in the Köppen classification), with moderately dry but extended summers (DRA-Centro, 1998). Mean annual temperature at the nearest weather station (Castelo Burgães: 40º 51'16"N, 8º 22'55"W at 306 m a.s.l) was 14.9 ºC. Annual rainfall at the nearest rainfall station (Ribeiradio: 40º 44'39"N, 8º18'05"W at 228m a.s.l) was, on average, 1609 mm, but varied markedly between 960 and 2530 mm (SNIRH, 2011 :1991-2011). Ermida belongs to the Hespheric Massif, one of the region's major physiographic units, which is mainly composed of pre-Ordovician schists and graywackes, although Hercynian granites do occur at several locations (Ferreira de Brum, 1978). The soils at the two study sites were described in the field and classified as Umbric Cambic Leptosols (Loamic), having shallow A-horizons of 13−20 cm overlying C− or R− horizons consisting of schists (IUSS, 2014).

Measurement of eroded sediments and collection of sediment and topsoil samples

At the end of August 2010, but before the starting of the rainy season, the two study sites were instrumented with a sediment fence (SF) at the bottom of the slope. The sediment fences or silt fences were made of geotextile fabric and supported by steel rods which were driven vertically into the soil, following the design of Robichaud and Brown (2002). While sediment fences let the overland flow through the mesh, they retain the eroded sediments with an estimated trapping efficiency of 68–93% (Spigel and Robichaud, 2007). The contributing area of the SF was estimated to be 192 m^2 in the case of the EB site and 89 m^2 in the case of the EU site.

Between the end of August 2010 and the end of August 2012, the eroded sediments retained by the SF were removed and weighted in the field at a total of 20 occasions. Large intact pieces of litter such as leaves and woody debris were removed. If the retained sediments accounted below < 0.5 kg, all of the material was taken to the laboratory; otherwise, one homogenized subsample was collected. These collected samples were analyzed for their moisture content by drying at 105^{o}C for 24h to compute sediment yield. The SF samples analyzed in this study were collected during mid-September 2010, immediately after the first significant rainfall events after the fire, as well as 5, 12 and 23 months later (Table 9). Concomitantly, the topsoils (0−2 cm depth)

of both study sites were sampled at 5 equidistant points along transects laid out from the bottom to the top of the slopes. The transects were marked to allow shifting their location 1 m across the slope on subsequent sampling occasions and, thereby, to avoid sampling spots disturbed earlier. Litter and, in the case of the EB site, also ashes were carefully removed before soil sample collection. After air-drying at room temperature, both the SF and the topsoil samples were sieved to the fine-earth fraction (< 2 mm), and stored under dark and cool conditions until further analyses (see 2.3). The yield of most SF samples from the unburnt site (SF−EU) was too small (< 5 g) to allow the various analyses, so that they were excluded from this study. Composite samples of the topsoil (one per site per sampling occasion) were obtained by thorough mixing of equal-weight sub-samples of the five soil samples collected at each site at each occasion.

Laboratory analysis of sediment and topsoil samples

Total nitrogen (TN) and total organic carbon (TOC) content of the samples were determined in triplicates by dry combustion using an elemental analyzer of the Perkin-Elmer 2400 series and a TOC Analyzer Shimadzu TOC−5050A respectively, whereas the pH values were measured with a glass electrode in the supernatant of a 1:2.5 weight/volume mixture of soil and distilled water in triplicates.

The sediment and composite topsoil samples were analysed by pyrolysis-gas chromatography–mass spectrometry (Py−GC/MS) using a vertical micro-furnace type double-shot pyrolyzer PY2020iD (Frontier Laboratories Ltd., Fukushima, Japan) attached to a GC/MS system (Agilent 6890 GC and Agilent 5973 MSD). Samples (ca. 1 mg) were introduced in the pre-heated micro-furnace at a pyrolysis temperature of 500 ºC for 1 min. The evolved gases from pyrolysis were transferred, through a capillary transfer line interface heated at 350 ºC, to the gas chromatograph inlet (splitless mode and 250 ºC). The gas chromatograph was equipped with a capillary column (J&W Scientific DB-5 MS) of 30 m × 250 µm × 0.25 µm film thickness. The oven temperature was held at 50 ºC for 1 min and then increased to 100 ºC at 30 ºC min^{-1}, from 100 ºC to 300 ºC at 10 ºC min^{-1}, and stabilized at 300 ºC for 10 min using a heating rate of 20 ºC min^{-1} in the scan mode. The carrier gas was helium at a controlled flow of 1 mL min^{-1} (De la Rosa et al., 2013). Electron ionization mass spectra were acquired with 70 eV ionising energy. Identification

of the individual compounds was carried out by using the extracted ion chromatograms for different homologous series and comparison with stored data (NIST and Wiley libraries). The peak areas of the different pyrolysis products were calculated as relative abundances based on percent peak areas of the total ion chromatogram trace (total integral of pyrolysis products identified equal to 100).

Data analyses

Principal Component Analysis (PCA) was performed by using the software Primer 6 (PRIMER-E Ltd, Plymouth, UK) on the normalized relative abundances of groups of compounds as identified by analytical pyrolysis and grouped according to their structure and origin.

Results and discussion

General parameters: total organic carbon, total nitrogen and pH

Two months after the wildfire, the EB topsoil still showed a greater pH than the EU topsoil (3.1 vs. 6.0; Table 9), which has been also observed in previous studies (Ulery et al., 1993; Certini et al., 2011; Granged et al., 2011; Martín et al., 2012; Pereira et al., 2012). This fact can be explained by the oxidation of the SOM and the fire-induced, loss of hydroxyl groups and the destruction of organic acids (Dumontet et al., 1996). In addition, the release of basic ions formerly bound in the litter contributed to liming effect of fires. Ulery et al. (1993) found a similar increase in topsoil pH of three units, immediately after burning. In laboratory experiments, Terefe et al. (2008) found that the pH increased significantly in soils with high soil organic matter contents and low clay contents following exposure to temperatures of 200−500ºC. Such, soil burning experiments under laboratory conditions, however, merely show the temperature on soil and do not include the liming effect of ashes. However, five months later, the pH of the EB topsoil dropped markedly (4.1) and remained practically constant until the end of the study although it was still higher than the pH of the EU topsoil. Arocena and Opio

(2003) found that fire-induced differences in pH values continued to exist three years after a wildfire. In contrast, Granged et al. (2011), found that the pH returned to pre-fire values between 3 and 6 months after a prescribed fire in an eucalyptus forest. The EU topsoil revealed a slight increase in pH throughout this study, possibly due to the deposition of ashes blown in from the burnt area.

Table 9 - Average pH, total organic carbon (TOC), total nitrogen (TN) and ratio TOC/TN of the sediment (SF) samples and of the topsoil (0–2 cm) at a recently burnt (EB) and a long unburnt (EU) eucalypt plantation on four sampling occasions.

Sampling period	Date of sediment collection	Months after fire	Code	pH (in H_20)	TOC ($g.kg^{-1}$)	TN ($g.kg^{-1}$)	TOC/N
I	21-Sep-10	2	SF-EB I	6.5	255	17	15
			EB I	6.0	154	14	11
			EU I	3.1	184	8	24
II	16-Feb-11	7	SF-EB II	5.3	255	15	17
			EB II	4.1	96	4	20
			EU II	3.2	127	2	34
III	14-Sep-11	14	SF-EB III	5.0	132	12	11
			EB III	4.2	97	5	17
			EU III	3.4	115	2	37
IV	22-Aug-12	25	SF-EB IV	4.2	312	12	27
			EB IV	4.0	84	3	18
			EU IV	3.5	118	4	25

The pH of the SF-EB after the fire decreased more gradually with time-since-fire than the pH of the EB topsoil but was consistently higher than for the EB topsoil at all four sampling periods. This is best explained by the admixture of ashes s.l. (sensu *lato*, i.e. including charcoal and partially charred plant and litter material) in the SF-EB. Shakesby et al. (2013) reported higher pH values in the sediments that had eroded four months following a prescribed fire in a shrub-dominated catchment in central Portugal than in the sediments that had eroded prior to the fire. The pH of these post-fire sediments, however, did not differ in a consistent manner from the pH of topsoil.

Two months after the wildfire, the TOC content of the topsoil from the burnt area were markedly lower than of the EU soils (154 vs. 184g kg^{-1} respectively). Such a fire-induced decrease in TOC content (of approx. 16 %) is in agreement with the findings of previous studies of Mediterranean soils (Gimeno-García et al., 2000; Mataix-Solera et al., 2002;

Certini et al., 2011; Granged et al., 2011; Badía-Villas et al., 2014b) and indicates that charcoal production did not compensate for the OM loss due to combustion (Knicker et al., 2006). The temporal pattern of the TOC contents at the EB site was similar to that of the pH values, reflecting a clear decrease (−38 %) by mid−February 2011 and then remaining basically unchanged until the end of the study. However, one has to bear in mind, that a similar tendency was also observed for the EU site which may indicate that this pattern is unrelated to the wildfire. While the post-fire losses in OC may, at least in part, have resulted from the removal of the protective litter and vegetation cover, the changes in TOC content at the EU site are probably related to inter-annual and variations in climatic conditions during the study period. Thus, the he considerably dry summer of 2008 the lead to a reduced biomass production and, thus, to a lower input of fresh OM into the soil.

The TOC contents at the EB sites were in accordance with previous studies by Campo et al. (2008), who attributed it to post-fire soil losses and slow vegetation recovery. Martins et al. (2013) and Prats et al. (2014) reported considerable erosion rates as well as reduced recovery of the ground vegetation for other recently burnt eucalypt plantations in the Ermida study area.

The SF-EB showed consistently higher TOC contents than the EB topsoil which was most likely related to the preferential transport of ashes *s.l.* by post-fire overland flow. The underlying assumption that the TOC content of the ashes and charcoal was higher than that of the topsoil is sustained by the findings of various studies (De la Rosa et al., 2008a; Santín et al., 2012; Bodí et al., 2014). With cumulative erosion and hence with increasing time-since-fire, it may be expected that the contribution of ash decreases due to a selective transport of ashes which leads to its exhaustion. Such a scenario, however, is not supported by the present results, since the highest TOC contents were determined for the SF-EB collected during the last sampling occasion. The exceptional high TOC contents of the SF-EB obtained from the last sampling event is best explained by a highly erosive event, which mobilized more sediments per mm of rainfall than any of the preceding events. Nonetheless, Malvar et al. (2013) also did not find clear evidence that OM losses in nearby eucalypt plantations decreased noticeably with time-since-fire during the first two years after fire.

On the first sampling occasion, the EB topsoil showed a 1.7–fold increase of TN as a result of the fire indicating that N-rich charcoal in which N is mostly bound in N-heterocyclic structures accumulated on the soil (Knicker et al., 2005; De la Rosa et al., 2008b). The temporal pattern of the topsoil TN contents at the two study sites were comparable but seem unrelated to the occurrence of the wildfire.

Comparable to the TOC, the TN content of the SF-EB was higher than that of the EB topsoil on all four sampling occasions, suggesting the same causes as underpin the fire-induced increases in topsoil TN contents. Unlike the TOC contents, however, the TN contents of the SF-EB did suggest a gradual decrease with time-since-fire.

The TOC/TN ratio diverged conspicuously between the EB and the EU site on the first sampling occasion (11 vs. 24) and was maintained throughout the entire study period. Such a fire-induced decrease in the C/N ratio has been observed in various studies (Fernández et al., 1999; González-Pérez et al., 2004; De la Rosa et al., 2008b) and could be due to the accumulation of newly-formed heterocyclic N forms.

Sediment losses

Cumulative sediment losses over the first two years after the wildfire were roughly 30 times lower at the EU site than at the EB site (Table 10). At the latter, the bulk of these losses (80%) occurred during the first seven months were produced by 44 % of the cumulative rainfall. The cumulative sediment losses at the EB plantation over the first post-fire year amounted to 1.1 Mg ha^{-1} y^{-1} (Table 10), closely matching the threshold value for tolerable soil losses of 1 Mg ha^{-1} y^{-1} proposed by Verheijen et al. (2009; 2012). The marked decrease in sediment losses over the second year agreed well with the commonly observed decrease in erosion response with time-since-fire (e.g. Shakesby and Doerr, 2006; Shakesby et al., 2013). These sediment losses during the first study year were higher than those reported by the majority of plot-scale erosion studies in the Mediterranean after moderate severity fires, as reviewed by Shakesby (2011).

Table 10 -- Cumulative sediment losses and cumulative rainfall amounts of the topsoil (0-2 cm) at a recently burnt (EB) and a long unburnt (EU) eucalypt plantation on four sampling occasions.

Monitoring period	Date of sediment collection	Cumulative sediment losses (kg.ha^{-1})		Cumulative rainfall (mm) Burnt (EB)
		Burnt (SF-EB)	Unburnt (SF-EU)	
I	21-Sep-10	9	0.3	25
II	16-Feb-11	1048	13	1119
III	14-Sep-11	1100	28	1472
IV	22-Aug-12	1306	46	2550

The four samples of SF-EB analyzed in this study were produced by the first rainfalls after the wildfire (amounting to 25 mm), by 108 mm of rainfall between mid−January and mid−February 2011, by 65 mm of rainfall between mid−August and mid−September 2011, and by 42 mm of rainfall between mid−June and mid−August 2012 (Fig. 17). In the case of the EU plantation, the amount of eroded sediments increased monotonically with increasing rainfall amount; in the case of the EB plantation, however, there was no obvious relationship between the amounts of eroded sediments and rainfall. Specific sediment losses at the EB were comparatively low for the winter−2011 sampling period and comparatively high for the summer−2012 sampling period (0.08/0.80 vs. 0.22−0.33 kg ha^{-1} mm^{-1} rainfall). Even so, the specific sediment losses at the EB site were at least twice as high as those at the EU site (0.01−0.05 kg ha^{-1} mm^{-1} rainfall) on all four sampling occasions.

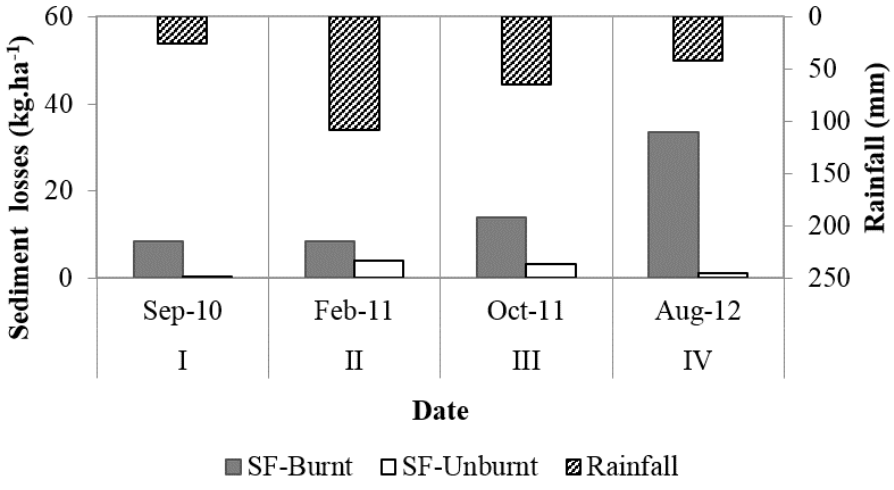

Figure 17 - 1 to 2-monthly sediment losses (SF) and corresponding rainfall amounts at a recently burnt (EB) and a long unburnt (EU) eucalypt plantation on four sampling occasions.

Organic matter composition in topsoil and eroded sediments

The pyrolysis products identified in the total ion current chromatograms (TIC) and their relative abundances for each of the individual samples are presented in Appendix A. The identified compounds were grouped according to their structure and origin into the following nine main groups: terpenoids; hydroaromatic steroids; *n*-alkanes/enes; *n*−fatty acid methyl esters (FAMEs); lignin−derived compounds; aromatic non−specific compounds; nitrogenated (N) compounds; polysaccharide−derived compounds and other compounds of unknown origin (unspecific).

The scatter plot of the first two principal components (PCs; Fig. 18), resumes the similarities in OM composition between the eroded sediments and (un−) burnt topsoil samples, and simultaneously shows the main groups of compounds responsible for these similarities. Together, the first two PC's accounted for 84% of the variability with the first PC (PC1) explaining 62% and the second PC (PC2) explaining 22%.

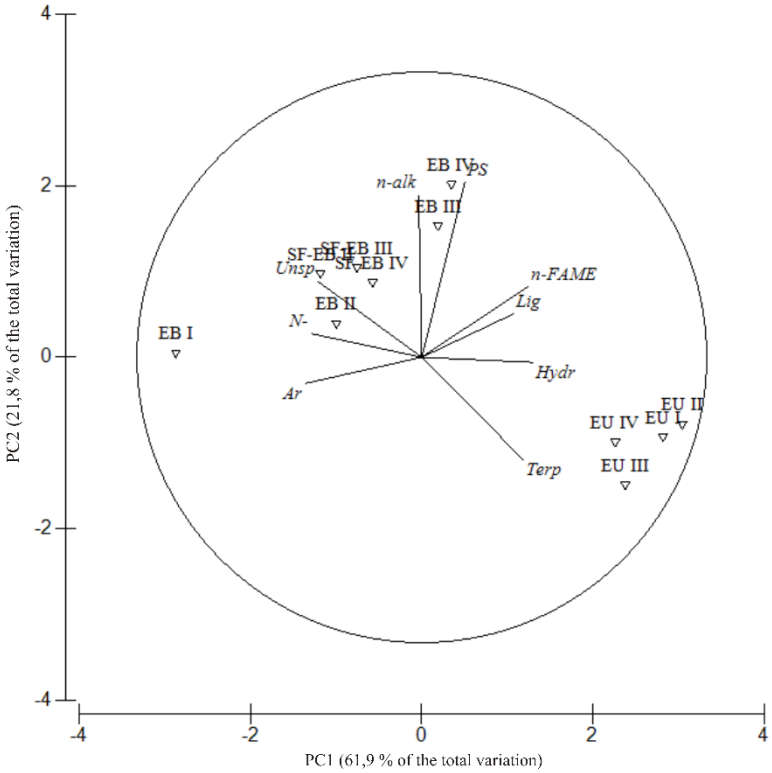

Figure 18 - Principal component analysis (PCA) of similarities in organic matter composition between the eroded sediments (SF–EB), burnt (EB) and unburnt topsoils (EU) and the main groups of compounds identified in Py–GC/MS. Terpenoids (terp); hydroaromatic steroids (Hydr); *n*-alkanes/enes (*n*-alk); *n*-fatty acidmethyl esters (*n*-FAMEs); lignin-derived compounds (Lig); aromatic non-specific compounds (Ar); nitrogenated compounds (N); polysaccharide-derived compounds (PS) and other compounds of unknown origin (Unsp).

The scatter plot allowed a good separation of the samples into three different sets. One set included the eroded sediments the first sampling period (SF-EB I), which showed a relatively strong affinity with aromatic non−specific compounds. The second set included the eroded sediments of the three following sampling occasions (SF-EB II, III and IV) as well as all burnt topsoil samples (EB I, II, III and IV), which had a similar composition in terms of N-compounds, *n*-alkanes/enes and polysaccharide−derived compounds. These results suggested that, seven months after the fire, the SF-EB had a similar composition as the EB topsoil. The third set include all unburnt topsoil samples (EU I, II, III and IV), which distinguished itself from the other samples through the presence of terpenoids and hydroaromatic steroids. Overall, PC1 was related to compounds such as *n*-FAMEs, polysaccharide−derived compounds, lignin−derived compounds, terpenoids and hydroaromatic steroids, while PC2 was associated with aromatic non−specific compounds, N−compounds and *n*-alkanes/enes, and polysaccharide−derived compounds.

The relative abundances of the above-mentioned nine main groups of identified compounds are presented in Fig. 19. The aromatic non−specific compounds were among the most abundant compounds in all samples. Their relative abundance was lower in the EU than EB topsoil and lower in the SF−EB samples, amounting to 29−33 %, 40−52 % and 44−69 %, respectively.

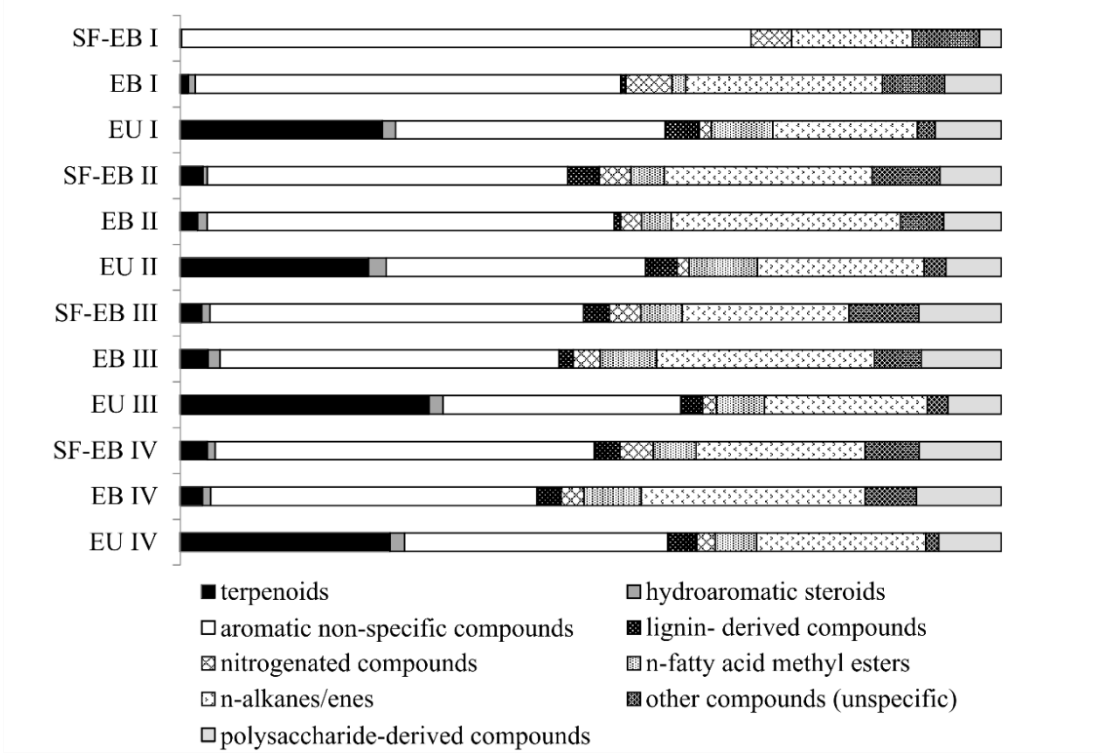

Figure 19 - Cumulative relative abundance of themajor types of pyrolysis products identified in eroded sediments (SF–EB), burnt (EB) and unburnt topsoils (EU) under eucalypt on the four periods in study. Period I: 2 months after fire; Period II: 7 months after fire; Period III: 14 months after fire and Period IV: 25 months after fire.

As it is shown by the PCA diagram, the detected aromatic compounds were mainly composed of alkyl benzenes (e.g. toluene, xylenes), polycyclic aromatic hydrocarbons (PAHs: indene, naphthalene, phenanthrene, antracene and biphenyl), and (methyl−) phenols. more abundant, as well as, more diverse in the EB and SF-EB samples than in the EU samples (Appendix A). Previous studies (e.g. González-Pérez et al., 2014) suggested that such compounds can be formed by incomplete combustion during wildfires and are indicative for the presence of charcoal. Accordingly, the PAHs were more abundant, as well as, more diverse in the EB and SF-EB samples than in the EU samples (Appendix A). These differences are most probably due to the presence of chars derived from biomass (Tinoco et al., 2006; Campo et al., 2011; De la Rosa et al., 2012; Badía-Villas et al., 2014b). It has been postulated that black carbon− i.e. carbonized substances produced during vegetation fires may be a major precursor of the aromatic substances present in burnt soils (Schmidt and Noack, 2000). The hydrophobic nature of charcoal residues might have contributed to the comparatively high erosion rates at the

EB site (Shakesby and Doerr, 2006; Malvar et al., 2013; Prats et al., 2014). Not only long unburnt but also recently burnt eucalypt plantations in the study region have been found to exhibit extreme topsoil water repellency, especially during dry antecedent weather conditions (Keizer et al., 2005; Keizer et al., 2008). Phenols and alkylphenols were found in all samples, probably originating from phenolic precursors compounds such as lignin, tannin, proteinaceous biomass, and carbohydrates (Tegelaar et al., 1995) or derived from the heat-transformed products of such constituents (Knicker et al., 2008).

Lignin−derived compounds were identified in all samples, except for the SF-EB samples collected at the first sampling occasion following the first post-fire rains of September 2010. The relative abundance of these lignin−derived compounds tended to be slightly lower in the EB samples (1−3 %) than in SF-EB samples (3−4 %), whereas that of EU samples was maintained constant (4%) during the study period. Among these compounds, the most abundant were 2−methoxy−4−vinylphenol, vanillin, vanillic acid methyl ester and acetovanillone, which probably derived from plants (Simoneit, 2002). In addition, the presence of 4-allyl-2-methoxyphenol (eugenol), was especially noteworthy as it is characteristic for woody materials (Sharma et al., 2004). Eugenol was identified in the Py−GC/MS chromatograms of earlier studies of Mediterranean forest soils (De la Rosa et al., 2008b; 2012; Knicker et al., 2013). In the present study, eugenol was detected in the EU samples but not in the EB, so that it could be a valid marker of fire occurrence in eucalypt plantations.

The principal forms of N−compounds identified in this study were hydrazine 1,1−dimethyl; pyridine and benzonitriles. The N−compounds tended to occur with a lower relative abundance in the EU samples (1−2 %) than in the EB (3−6 %) as well as SF-EB samples (4−5 %). This behavior was consistent with that observed for the TN contents. Furthermore, it was in accordance with the increases in heterocyclic N−compounds that was associated with the alteration of SOM by fire (De la Rosa et al., 2008b; Knicker, 2010).

Polysaccharide-derived compounds were present in all pyrolysates of the soil and sediment samples collected in this study. They are considered to originate from plant, fungal and/or microbial materials. They consist of furans, lactones (furanones), cyclic

ketones (cyclopentanones) and anhydrosugars (Gregorich et al., 1996; Yassir and Buurman, 2012). The most abundant polysaccharide-derived compounds in the studied samples were furans (furan, 3−methyl−; benzofuran; 2−furancarboxaldehyde, 5−methyl−; furan, 2−(1−pentenyl)−; 1H−Inden−1−one, 2,3−dihydrotetramethyl−), anhydrosugars (d−allose) and furfural. The relative abundance of the polysaccharide-derived compounds attained its lowest value in the eroded sediments (SF−EB: 3−10 % vs. EU: 7−8 % and EB: 7−10 %). The compound D-allose (corresponding to peak 140, Appendix A) probably originated to a large extent from polysaccharides that have been thermally altered (Poirier et al., 2005). Its presence solely in the EU could indicate that the wildfire-induced soil heating transformation of some polysaccharidic compounds.

Terpenoids− which are commonly used as plant biomarkers− occurred with higher relative abundance in the EU samples (23−30 %) than in both the EB (1−3 %) and SF−EB samples (<1−3 %), as it is also shown by the PCA diagram (Figure 18). This suggested that the wildfire had a drastic impact on plant cover, and produced a noticeable change in the SOM molecular composition. Eucalyptol and globulol –which are indicators for the presence of *Eucalyptus globulus* (Song et al., 2009; de Blas et al., 2013)− attained relative abundances of 2 % and 1 %, respectively, in EU samples, but occurred less frequently and with a lower contribution in both the EB and SF-EB samples. Only the EB sample taken mid-September 2011 contained very low amounts of eucalyptol and globulol (0.3 %). None of the SF-EB samples revealed globulol, but several contained eucalyptol from mid-February 2011 onwards, albeit with low relative abundances which decreased with time-since-fire from 0.5 to 0.3 %. The appearance of globulol in the SF-EB, some six months after the fire and its subsequent decrease in relative abundance with time-since-fire suggested input from litter fall from scorched eucalypt crowns and/or from post-fire logging rather than input from litter fall from resprouting eucalypt.

Hydroaromatic steroids were found in the pyrochromatograms of all topsoil samples but with lower relative abundances in SF-EB and EB than in EU (c. 1 vs. 2 %). Furthermore, they occurred with similar relative abundances in the pyrograms of SF-EB and of EB (≈1 %), except of those from the first sampling. In contrast, β-sitosterol, occurred with higher relative abundances in SF-EB and EB than EU (0.1–0.3% vs. 0.1%). This compound

is indicative for plant residues since it is one of the most common steroids in the wax and gums of higher plants (Baker, 1982).

The doublets of *n*-alkanes/*n*-alkenes series represented an important fraction of the pyrolysis products, ranging from 15 to 27 % of the total integrated area of the Pyrochromatogram (Fig. 20). Their C numbers varied from C_9 to C_{31}, and showed bimodal distributions whose maxima partially overlapped for the different types of samples (Fig. 5). These overlapping maxima coincided with the ranges of C_{13} to C_{15} (EU: C_{15}; EB: C_{13}-C_{15}; SF-EB: C_{13}, C_{15}) and C_{21} to C_{22} (EU: C_{21}, C_{22}; EB: C_{22}; SF-EB: C_{21}). In addition, the pyrogram of EU showed a third maximum at C_{29}.

The typical molecular ratios for the *n*-alkanes are given in Table 11. Compared to OM of the EU samples, that of the EB samples lose the odd-over-even predominance (Carbon Preference Indexes−CPI), a reduction in the Average Chain Length (ACL), and an increase in the ratio of short-to-long *n*-alkanes ($\sum C_9$-$C_{22}/\sum C_{23}$-C_{31}).

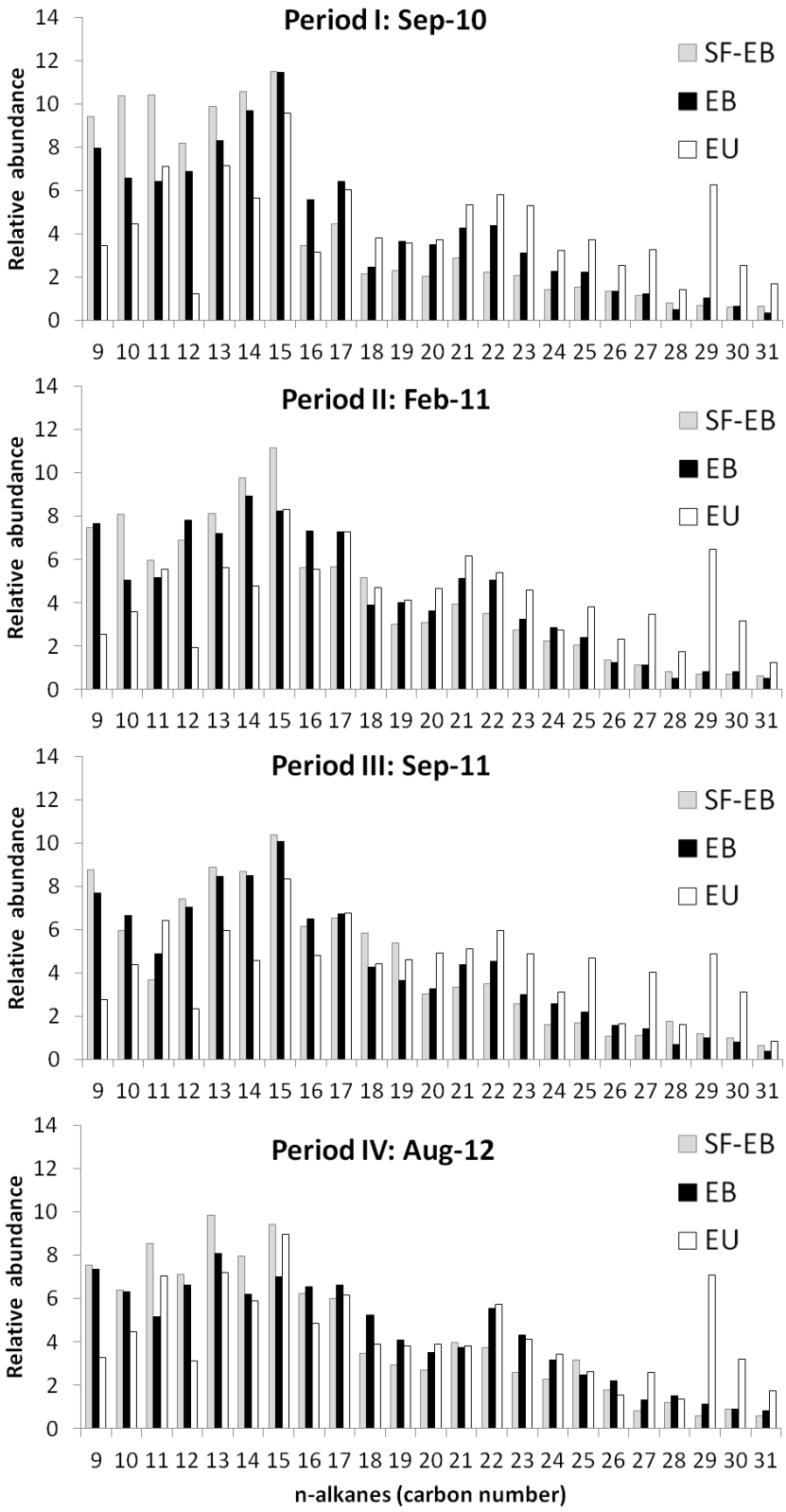

Figure 20 - Relative abundance of *n*-alkanes/enes in the eroded sediments (SF–EB), burnt (EB) and unburnt (EU) topsoils under eucalypt plantation on four sampling occasions. Period I: 2 months after fire; Period II: 7 months after fire; Period III: 14 months after fire and Period IV: 25months after fire.

Table 11 -- Molecular ratios for *n*-alkanes in the sediment (SF), burnt (EB) and unburnt (EU) topsoils under eucalypt plantation on four samples occasions

	$\sum C_9$-$C_{22}/\sum C_{23}$-C_{31} short/long			**Diff.**		CPI (odd/even)			**Diff.**		CPI short			**Diff.**		CPI long			**Diff.**		ACL			**Diff.**	
Periods	SF-EB	EB	EU	**SF vs. EB**	**EB vs. EU**	SF-EB	EB	EU	**SF vs. EB**	**EB vs. EU**	SF-EB	EB	EU	**SF vs. EB**	**EB vs. EU**	SF-EB	EB	EU	**SF vs. EB**	**EB vs. EU**	SF-EB	EB	EU	**SF vs. EB**	**EB vs. EU**
I	8.8	6.9	2.3	**1.8**	**4.6**	1.3	1.3	1.7	**0**	**-0.4**	1.3	1.2	1.5	**0.1**	**-0.3**	1.5	1.7	2.1	**-0.2**	**-0.4**	14.7	17.2	18.6	**-2.5**	**-1.4**
II	7.1	6.4	2.4	**0.7**	**4.0**	1.1	1.1	1.5	**0**	**-0.4**	1.1	1.1	1.3	**0**	**-0.2**	1.4	1.5	2.0	**-0.1**	**-0.5**	16.0	16.3	19.1	**-0.3**	**-2.8**
III	6.9	6.4	2.5	**0.5**	**3.9**	1.2	1.2	1.5	**0**	**-0.3**	1.2	1.1	1.3	**0.1**	**-0.2**	1.3	1.4	2.0	**-0.1**	**-0.6**	16.0	16.1	18.7	**-0.1**	**-2.5**
IV	6.2	4.6	2.6	**1.6**	**2.0**	1.3	1.1	1.4	**0.2**	**-0.3**	1.3	1.1	1.3	**0.2**	**-0.2**	1.2	1.3	1.9	**-0.1**	**-0.6**	15.9	16.9	18.5	**-1.0**	**-1.5**

Average chain length = $(\sum Z_n \times n)/\sum Z_n$), with Z_n as relative amount of *n*-alkanes with *n* carbons.

Carbon preference index of *n*-alkanes = $(\sum C_{9\text{-}31odd}/\sum C_{10\text{-}30even})$

Carbon preference index of short chain *n*-alkanes = $(\sum C_{9\text{-}21odd}/\sum C_{22\text{-}30even})$

Carbon preference index of long chain *n*-alkanes = $(\sum C_{9\text{-}31odd}/\sum C_{22\text{-}30even})$

Diff. = difference between sediment –burnt (SF vs. EB) and burnt-unburnt topsoil (EB vs. EU)

The differences in *n*-alkanes molecular ratios between the SF-EB and EB samples were most pronounced for the samples of the first sampling occasion, for which an ACL of 14.7 and 17.2 and a short-to-long ratio of 8.8 and 6.9 were determined. The difference of both indicators was smaller for the material collected at the next two sampling occasions but then increased again for that of the last sampling occasion. The latter reflected first and foremost a change in the properties of the burnt topsoil. By mid-august 2012, two years after the fire, the ACL and short-to-long ratio of the EB closely approximated the values of the EU, thus suggesting a perceptible recovery from the direct fire effects.

Fatty acids (FA) were found with rather low relative abundances in the majority of samples, typically ranging from 2 and 8 %. Furthermore, they were absent in all SF−EB samples. However, analytical pyrolysis is expected to underestimate FA detection due to incompatibility of FA with non-polar columns used in GC as well as due to their decarboxylation at elevated temperatures (Bahri et al., 2006).

Conclusions

Two years after the fire partial recovery of the soil properties were indicated. The eroded sediments after the fire were consistently more acid and richer in TOC and TN than the burnt topsoil, and also revealed less pronounced changes with time-since-fire, which confirmed that the loss of SOM in quality and quantity occurred during the two years monitored.

Analytical pyrolysis of topsoil and sediment samples exposed that fire effects at the burnt site involved:

(i) a comparative enrichment of the topsoil in aromatic compounds and especially, PAHs derived from charcoal and partially charred OM as well as an additional enrichment of the eroded sediments, which should be duly considered due to its abundance of persistent organic pollutants.

(ii) increases in heterocyclic N together with an increase in the short-to-long ratio of *n*-alkanes, and reduction in average chain length, which all indicated thermal transformation of the fire affected SOM.

(iii) a decrease of biomarkers of *Eucalyptus globulus*, as well as in other terpenoids used as specific biomarkers of vegetation, which is consistent with noticeable fire-induced changes in vegetation cover.

In addition, the Py−GC/MS results indicated that the recovery of the topsoil SOM quality from the immediate fire effects was partial during the two years covered by this study.

Finally, the present study showed that moderate-severity wildfire considerably increased the sediment losses during the first year after the wildfire such that they exceeded the well-established threshold for tolerable soil losses of 1 Mg ha^{-1} y^{-1}. Therefore, the long-term effects of fire and post-fire erosion on SOM quality would deserve further research attention, especially through a chrono-sequence of time-since-fire. The long-term recovery of SOM is particularly relevant from a management point-of-view, given the crucial role of SOM in the key ecosystem services provided by eucalypt plantations such as biomass production and carbon sequestration as well as the widespread occurrence of eucalypt plantations in Portugal and across the globe.

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Appendix A – Supplementary data

Appendix A - Py-GC/MS compounds identified in the sediment (SF–EB) samples and burnt (EB) and unburnt (EU) topsoil on the four periods in study.Period I: 2 months after fire; Period II: 7 months after fire; Period III: 14 months after fire and Period IV: 25 months after fire.

Peak label	*n*-alkanes/enes	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
8	Octene	C_8H_{16}	1.6	0.4	0.6	0.6	0.6	0.2	0.1	0.2	-	-	-	0.7
13	Nonene	C_9H_{18}	1.6	0.9	0.5	0.6	0.9	0.9	0.7	0.7	0.4	0.5	0.3	0.8
19	Decene	$C_{10}H_{20}$	1.9	2.3	2.2	1.4	2.2	1.8	1.5	1.0	0.8	0.8	0.7	1.4
36	Undecane	$C_{11}H_{24}$	0.7	-	-	-	-	-	-	-	-	-	-	-
37	Undecene	$C_{11}H_{22}$	-	2.5	2.1	1.1	0.4	2.3	2.2	2.5	0.6	0.7	0.9	0.8
62	Dodecane	$C_{12}H_{26}$	0.7	-	-	0.7	-	-	0.8	0.9	-	-	-	1.4
63	Dodecene	$C_{12}H_{24}$	0.7	1.6	1.4	0.7	1.8	1.9	0.8	0.9	0.8	0.7	0.8	-
83	Tridecane	$C_{13}H_{28}$	0.4	-	-	0.9	1.6	0.8	0.8	0.8	-	0.7	0.5	-
84	Tridecene	$C_{13}H_{26}$	-	1.5	1.3	0.9	-	0.8	0.8	0.8	1.2	0.7	0.5	1.4
99	Tetradecane	$C_{14}H_{30}$	0.7	0.8	0.8	0.7	-	0.9	0.9	0.9	-	-	-	-
100	Tetradecene	$C_{14}H_{28}$	0.3	0.8	0.8	0.7	1.7	0.9	0.9	0.9	1.2	1.7	1.1	1.3
122	Pentadecane	$C_{15}H_{32}$	0.7	-	0.8	0.8	-	0.9	1.0	0.9	0.9	0.9	0.6	0.8
123	Pentadecene	$C_{15}H_{30}$	0.2	1.7	0.8	0.8	1.8	0.9	1.0	0.9	0.9	0.9	-	0.8
141	Hexadecane	$C_{16}H_{34}$	0.3	0.6	0.5	0.4	-	0.7	0.7	0.8	-	-	-	-
142	Hexadecene	$C_{16}H_{32}$	0.1	0.6	0.5	0.4	1.1	0.7	0.7	0.8	0.8	0.8	0.7	0.9
157	Heptadecane	$C_{17}H_{36}$	0.6	0.7	0.6	0.5	-	0.8	0.7	0.6	-	0.3	0.7	1.1
158	Heptadecene	$C_{17}H_{34}$	0.2	0.7	0.6	0.5	1.6	0.8	0.7	0.6	0.7	0.3	-	-
170	Octadecane	$C_{18}H_{38}$	0.1	0.2	0.5	0.6	0.6	0.7	0.6	0.6	-	0.3	0.4	0.5
171	Octadecene	$C_{18}H_{36}$	-	1.0	0.5	0.6	0.6	0.7	0.6	0.6	0.5	0.3	0.4	0.5
174	Nonadecane	$C_{19}H_{40}$	0.5	0.4	0.5	0.4	0.5	0.6	0.5	0.6	-	-	-	-
175	Nonadecene	$C_{19}H_{38}$	-	0.4	0.5	0.4	0.5	0.6	0.5	0.6	1.1	0.9	0.8	0.6
186	Eicosane	$C_{20}H_{42}$	0.3	0.7	0.3	0.4	0.5	0.7	0.7	0.4	-	0.5	0.4	0.4
187	Eicosene	$C_{20}H_{40}$	-	0.7	0.3	0.7	0.5	0.7	0.7	0.8	1.1	0.5	0.5	0.4
194	Heneicosane	$C_{21}H_{44}$	0.4	0.5	0.2	0.3	0.6	0.5	0.4	0.8	-	0.5	0.4	0.4
195	Heneicosene	$C_{21}H_{42}$	0.2	0.7	0.4	0.4	0.7	0.9	0.9	0.8	0.8	0.5	0.4	0.4
198	Docosane	$C_{22}H_{46}$	0.3	0.3	0.2	0.3	0.5	1.5	1.4	1.6	0.7	1.0	0.9	0.9
199	Docosene	$C_{22}H_{44}$	0.3	1.8	1.1	1.1	1.7	1.5	1.4	1.6	0.7	1.0	0.9	0.9

(continuation)

Peak label	*n*-alkanes/enes	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
201	Tricosane	$C_{23}H_{48}$	0.3	0.4	0.2	0.2	0.4	0.4	0.3	0.3	-	0.2	1.8	0.2
202	Tricosene	$C_{23}H_{46}$	0.2	0.4	0.2	0.3	0.4	0.5	0.5	0.4	1.7	0.4	1.8	0.2
205	Tetracosane	$C_{24}H_{50}$	0.2	0.2	0.1	0.2	0.3	0.3	0.4	0.5	0.3	0.5	0.4	0.4
206	Tetracosene	$C_{24}H_{48}$	0.2	0.9	0.5	0.6	0.8	1.0	1.1	1.0	0.3	0.5	0.4	0.4
211	Pentacosane	$C_{25}H_{52}$	0.2	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.1
212	Pentacosene	$C_{25}H_{50}$	0.1	0.3	0.2	0.2	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2
215	Hexacosane	$C_{26}H_{54}$	0.3	0.2	0.1	0.3	0.2	0.2	0.3	0.3	0.4	0.6	0.4	0.4
216	Hexacosene	$C_{26}H_{52}$	0.2	0.6	0.4	0.3	0.5	0.6	0.3	0.3	0.4	0.6	0.4	0.4
218	Heptacosane	$C_{27}H_{56}$	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.2	0.2
219	Heptacosene	$C_{27}H_{54}$	-	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.1	0.2	0.2	0.2
220	Octacosane	$C_{28}H_{58}$	0.1	0.1	-	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.3	0.1
221	Octacosene	$C_{28}H_{56}$	-	0.1	0.4	0.3	0.2	0.1	0.1	0.2	0.3	0.7	0.3	0.5
222	Nonacosane	$C_{29}H_{60}$	-	-	-	0.1	-	-	-	0.2	0.2	0.4	0.2	0.3
223	Nonacosene	$C_{29}H_{58}$	-	-	-	0.1	-	-	-	0.1	0.1	0.1	0.1	0.1
225	Triacontane	$C_{30}H_{62}$	-	-	-	0.2	-	-	-	-	0.1	0.2	0.2	-
226	Triacontene	$C_{30}H_{60}$	-	0.1	0.1	0.2	-	-	0.2	0.2	0.2	0.5	0.5	0.5

(continuation)

Peak label	*n*-FAMEs	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
42	2-Butenoic acid, 2-methyl-	$C_5H_8O_2$	-	-	-		-	-	-	-	0.8	0.4	1.5	0.7
69	Heptanoic acid	$C_7H_{14}O_2$	-	-	-	-	-	-	-	-	0.3	-	0.3	0.7
76	Octanoic acid	$C_8H_{16}O_2$	-	-	-	0.5	-	-	-	-	-	-	-	
97	Butanoic acid, 3-methyl-, 2-methylbutyl ester	$C_{10}H_{20}O_2$	-	1.6	2.1	2.4	0.7	1.2	1.3	2.0	-	-	-	-
108	Benzenebutanoic acid, α-methyl-γ-oxo- methyl-	$C_{11}H_{12}O_3$	-	0.2	1.1	-	-	-	-	-	-	-	-	-
163	Propanoic acid, 2-methyl-, nonyl ester	$C_{13}H_{26}O_2$	-	1.1	1.1	1.0	0.7	1.0	1.3	1.2	1.1	1.4	1.1	0.9
178	Tetradecanoic acid	$C_{14}H_{28}O_2$	-	-	-	0.3	-	-	0.6	0.8	0.9	1.4	0.3	-
190	FAME $C_{16:1}$	$C_{17}H_{32}O_2$	-	0.2	0.1	0.2	-	0.2	0.2	0.2	-	-	-	-
196	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	-	0.6	0.3	0.7	-	0.7	1.9	2.1	2.3	2.8	2.3	1.8
203	Oleic acid	$C_{18}H_{34}O_2$	-	0.2	0.2	-	-	-	1.0	-	1.3	1.4	-	0.8
204	Octadecanoic acid	$C_{18}H_{36}O_2$	-	-	-	-	-	-	-	-	0.7	0.8	0.4	-
214	FAME C_{22}	$C_{23}H_{46}O_2$	-	-	-	-	-	0.1	0.2	0.3	-	-	-	-
217	Erucic acid	$C_{22}H_{42}O_2$	-	0.3	0.2	0.3	0.2	0.5	0.4	0.5	0.1	0.2	0.1	0.2

Peak label	Aromatic compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
4	Benzene	C_6H_6	9.3	2.1	1.2	1.1	1.6	1.2	1.3	1.0	0.3	0.4	0.5	0.4
9	Toluene	C_7H_8	12.2	5.0	5.9	4.4	6.1	4.2	4.5	3.9	3.1	3.3	2.2	2.5
12	Cresol	C_7H_8O	-	0.2	0.6	0.6	-	0.5	0.2	0.5	0.4	0.2	-	-
14	Ethylbenzene	C_8H_{10}	3.4	0.7	0.7	0.8	0.6	0.9	0.3	0.2	-	-	-	0.3
15	p-Xylene	C_8H_{10}	4.2	2.1	1.5	1.3	3.6	3.2	2.4	2.4	1.1	1.7	1.8	1.5
16	Styrene	C_8H_8	2.1	1.5	3.3	3.5	2.0	2.2	1.3	2.9	2.6	2.1	0.8	1.3
20	Benzene. 1-ethyl-3-methyl-	C_9H_{12}	3.1	1.6	2.0	2.7	2.4	3.2	3.0	3.6	1.0	0.4	0.6	1.5
27	1-(2-Methylphenyl)ethanol	$C_9H_{12}O$	-	0.6	0.2	0.2	-	-	-	-	-	-	-	-
28	Benzene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{14}$	4.7	1.6	1.4	1.2	3.2	3.1	1.2	0.6	1.9	1.2	3.1	1.9
30	Benzene, 1,2,3-trimethyl-	C_9H_{14}	1.6	-	-	-	-	-	-	-	0.4	0.1	0.3	-
31	Benzaldehyde	C_7H_6O	0.7	-	0.8	0.4	-	2.0	0.5	0.8	-	-	-	0.6

(continuation)

Peak label	Aromatic compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
33	Acetophenone	C_8H_8O	-	2.3	0.5	1.0	2.8	0.7	-	-	-	-	-	0.6
44	Benzene, butyl-	$C_{10}H_{14}$	0.6	-	-	-	-	-	-	-	-	-	-	-
47	1H-Indene	C_9H_8	-	0.6	-	0.5	0.6	0.6	0.8	0.8	0.1	0.3	0.5	0.6
50	o-isopropenyltoluene	$C_{10}H_{12}$	2.8	0.6	0.9	0.7	1.5	1.5	1.0	0.5	0.9	0.9	1.0	1.1
52	benzeneacetaldehyde	C_8H_8O	-	-	-	-	-	0.3	-	-	0.3	0.4	-	-
54	Benzaldehyde, 3-hydroxy-	$C_7H_6O_2$	-	0.4	0.8	0.8	-	-	-	-	-	-	-	0.6
58	Benzene, ethyl-1,2,4-trimethyl-	$C_{11}H_{16}$	-	-	-	-	-	-	-	-	-	-	-	0.4
59	Phenol	C_6H_6O	-	2.2	3.6	3.4	3.2	2.7	1.5	1.3	1.1	0.6	0.6	0.5
60	Acetophenone	C_8H_8O	0.6	1.0	1.5	1.5	0.9	1.5	1.5	1.8	1.3	1.0	0.5	2.2
64	Benzene, pentyl-	$C_{11}H_{16}$	4.5	0.4	0.4	0.4	0.6	0.5	0.5	-	-	-	-	0.3
65	Benzene, (1-methyl-2-propeny)oxy)	$C_{10}H_{12}O$	1.7	-	-	-	-	-	-	-	-	-	-	-
66	Phenol, 2-methoxy-	$C_7H_8O_2$	-	1.1	2.3	2.5	0.8	0.9	1.2	1.6	2.8	2.6	1.7	1.7
67	Benzene, 1,2,3,4-tetramethyl	$C_{10}H_{14}$	1.2	0.5	0.4	0.5	0.7	0.8	1.0	0.4	-	-	-	-
68	Phenol, 2-methyl-	C_7H_8O	-	-	-	-	-	-	-	-	0.7	0.9	0.4	-
71	2-Methylindene	$C_{10}H_{10}$	-	0.4	0.4	0.4	0.9	0.4	0.4	0.4	-	-	-	-
72	Benzene, 1-methyl-4-(1-methyl-2-propenyl)-	$C_{11}H_{14}$	-	-	-	-	-	-	-	-	0.3	0.3	0.4	0.6
73	Phenol, 4-methyl-	C_7H_8O	-	0.7	0.9	0.8	-	0.4	0.6	0.4	2.7	2.5	1.7	2.0
74	1,2-Dihydronaphthalene	$C_{10}H_{10}$	-	-	-	-	-	-	0.6	0.5	-	-	-	-
75	Tetramethyl benzene	$C_{10}H_{14}$	-	-	-	-	-	-	0.3	0.3	-	-	-	-
77	1,2,3,4-Tetrahydro-1-naphthalenemethanol	$C_{11}H_{14}O$	-	0.7	0.5	-	0.6	0.5	0.6	0.6	-	-	-	-
79	Phenol, 4-methyl-	C_7H_8O	-	1.1	2.0	2.5	1.7	0.8	1.0	1.1	-	-	-	-
80	2,2-Dimethylindene, 2,3-dihydro-	$C_{11}H_{14}$	-	-	-	-	-	0.9	0.8	0.8	-	-	-	-
82	Naphthalene	$C_{10}H_8$	1.5	1.1	1.0	0.6	1.6	1.0	1.0	0.9	-	-	0.4	1.0
86	Benzene, hexyl-	$C_{12}H_{18}$	0.6	1.1	0.3	-	0.3	-	-	-	-	-	-	-
88	2H-1-Benzopyran, 3,4-dihydro-2,2-dimethyl-	$C_{11}H_{14}O$	0.4	-	-	-	0.7	0.1	0.4	0.4	-	-	-	-
89	Phenol, 2-methoxy-4-methyl-	$C_8H_{10}O_2$	-	0.8	1.0	0.8	0.5	0.6	0.8	0.7	1.0	0.8	0.5	0.5
90	Phenol, 2,3-dimethyl-	$C_8H_{10}O$	-	0.6	0.6	0.3	0.3	0.7	0.6	0.5	0.6	0.6	0.4	0.3
91	1-(3-Methylphenyl)buta-1,3-diene	$C_{11}H_{22}$	-	-	-	-	-	-	-	-	0.3	0.3	0.2	0.2
92	1H-Indene, 1,3-dimethyl-	$C_{11}H_{12}$	-	0.4	0.2	0.2	0.7	0.7	1.0	0.4	0.5	0.4	0.3	0.2

(continuation)

Peak label	Aromatic compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
94	Naphthalene, 1,2-dihydro-3-methyl-	$C_{11}H_{12}$	0.9	0.2	-	-	0.2	0.3	0.2	0.2	-	-	-	-
95	Phenol, 4-ethyl-	$C_8H_{10}O$	0.2	1.0	0.9	1.5	0.7	1.3	1.3	1.4	1.5	1.3	1.0	1.2
101	Phenol, 4-ethyl-2-methoxy-	$C_9H_{12}O_2$	-	-	-	-	-	-	-	-	0.8	-	0.5	-
102	Benzene, 1,2-bis(1-methylethyl)-	$C_{12}H_{18}$	0.6	1.4	1.8	1.8	1.9	0.5	0.5	0.4	-	0.7	1.2	2.3
105	Naphthalene, 1,2,3,4-tetrahydro-1,1,6-trimethyl-	$C_{13}H_{18}$	-	0.2	-	1.3	0.9	0.2	0.5	0.6	-	-	-	0.6
106	2-Cyclopenten-1-ol, 1-phenyl-	$C_{11}H_{12}O$	-	-	-	-	-	0.9	-	-	-	-	-	-
107	Benzene, heptyl-	$C_{13}H_{20}$	0.3	1.0	1.1	-	0.4	-	-	-	-	-	-	-
111	2,3-Dihydro-benzofuran	C_8H_8O	-	-	-	0.7	-	0.4	0.4	0.7	0.9	0.5	0.8	0.9
118	Phenol, m-tert-butyl-	$C_{10}H_{14}O$	-	0.6	-	-	-	0.5	0.8	0.4	-	-	-	-
119	Biphenyl	$C_{12}H_{10}$	1.2	0.2	0.3	0.7	1.9	0.4	0.4	0.3	-	-	-	-
120	Benzene, 1,4-bis(1-methylethenyl)-	$C_{12}H_{14}$	0.3	0.4	0.2	-	-	0.4	0.2	0.2	-	-	-	-
125	Phenol,2,6-dimethoxy	$C_8H_{10}O_3$	-	-	-	-	-	-	-	-	0.5	0.6	0.5	-
127	1,1,6,8-Tetramethyl-1,2-dihydro-naphthalene	$C_{14}H_{18}$	0.8	-	-	-	-	-	-	-	-	-	-	-
128	Benzene, octyl-	$C_{14}H_{22}$	0.2	0.5	0.2	-	0.6	0.5	0.6	0.5	-	0.9	-	-
130	Naphthalene, 2,7-dimethyl-	$C_{12}H_{12}$	0.4	1.4	0.9	1.0	1.4	0.9	0.9	0.7	-	-	-	0.7
135	Naphthalene, 1,2,3,4-tetrahydro-2,2,5,7-tetramethyl-	$C_{14}H_{20}$	-	0.1	0.2	0.3	0.2	0.8	0.6	0.3	-	-	-	-
143	Phenol, 2-methoxy-4-(1-propenyl)	$C_{10}H_{12}O_2$	-	-	-	-	-	-	-	-	0.3	0.4	-	-
144	Benzene, (2-ethyl-4-methyl-1,3-pentadienyl)	$C_{14}H_{18}$	-	-	-	-	-	-	-	-	0.5	0.9	0.8	-
146	Benzene, nonyl-	$C_{15}H_{24}$	0.3	0.2	-	0.1	0.4	0.3	0.2	0.3	-	-	-	-
150	Naphthalene, 2,3,6-trimethyl-	$C_{13}H_{14}$	-	-	-	-	-	-	0.7	0.3	-	-	-	-
151	Naphthalene, 1-methyl-7-(1-methylethyl)-	$C_{14}H_{16}$	0.1	0.9	0.6	-	0.8	1.6	0.8	0.5	0.3	0.3	0.4	0.3
152	(2E)-2-(4-Methoxybenzylidene) cyclohexanone	$C_{14}H_{16}O_2$	-	-	-	-	-	-	-	-	0.3	0.2	0.4	0.3
153	2H-1-Benzopyran-2-one	$C_9H_6O_2$	-	-	0.3	0.4	-	-	-	-	-	-	-	-
154	Naphthalene, 1,4,6-trimethyl-	$C_{13}H_{14}$	2.0	0.4	0.5	0.9	-	0.4	0.8	-	0.2	0.4	0.9	-
155	Naphthalene, 2,3,6-trimethyl-	$C_{14}H_{16}$	-	-	-	-	0.7	0.2	0.2	0.4	-	-	-	-
159	1,2,3,4,5,6,7,8-Octahydro-1-methylphenanthrene	$C_{15}H_{20}$	-	-	-	-	-	-	-	-	-	-	0.3	-
162	Benzene,3,5-dimethyl-1-(phenylmethyl)	$C_{15}H_{16}$	2.6	-	-	-	0.2	-	-	-	0.4	0.8	0.3	0.1
164	9H-fluorene	$C_{13}H_{10}$	-	0.3	0.3	0.3	0.2	-	-	-	-	-	-	-
166	3,7-Benzofurandiol, 2,3-dihydro-2,2-dimethyl-	$C_{10}H_{12}O_3$	-	-	-	-	-	-	-	-	1.0	0.8	1.0	-

(continuation)

Peak label	Aromatic compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
168	Phenol, 4-(1-methyl-1-phenylethyl)- (CAS)	$C_{15}H_{16}O$	0.3	0.3	0.3	0.2	1.1	0.5	-	-	0.3	0.5	0.3	0.6
169	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	$C_{15}H_{18}$	2.2	0.7	1.4	1.1	1.3	0.7	0.4	0.3	0.8	0.5	1.0	0.3
173	7-isopropyl-4-methylazulene	$C_{14}H_{16}$	0.5	-	-	-	0.5	0.4	-	-	-	-	-	-
176	Isopropylbiphenyl	$C_{15}H_{16}$	0.4	-	-	-	-	-	-	-	-	-	-	-
177	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	$C_{15}H_{18}$	-	-	-	-	-	-	-	-	0.1	0.3	0.5	0.2
180	1H-Indene, 2-decyloctahydro-	$C_{19}H_{36}$	0.2	0.3	0.4	0.1	-	0.3	0.3	0.4	-	0.2	0.2	0.3
181	Anthracene, 1,2,3,4,5,6,7,8-octahydro-	$C_{14}H_{18}$	-	0.1	0.4	0.1	-	-	0.3	0.3	-	-	-	-
183	9H-Fluoren-9-one	$C_{13}H_8O$	-	-	0.5	0.4	-	0.2	0.3	0.4	-	-	-	-
184	Phenanthrene	$C_{14}H_{10}$	-	0.7	-	-	0.4	-	-	-	-	-	-	0.2
185	Ethanone, 1-(4-hydroxy-3,5-dimethoxyphenyl)-	$C_{10}H_{12}O_4$	-	-	-	-	-	-	-	-	0.4	0.3	0.2	-
191	Anthracene, 1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl-	$C_{16}H_{22}$	-	-	-	-	-	-	-	-	0.2	0.3	0.2	-
192	Antracene	$C_{14}H_{10}$	0.2	-	-	-	-	-	-	-	-	-	-	-
193	9,10- Phenanthrenedione	$C_{14}H_8O_2$	0.4	-	-	-	-	-	-	-	-	-	-	-
197	1,2,3,4,4a,10a-Hexahydro-1,1,4a-trimethyl-7-(1'-methylethyl)phenanthrene	$C_{20}H_{28}$	-	0.5	0.1	0.2	-	-	-	-	-	-	-	-
200	Anthracene, 9,10-dimethoxy-	$C16H_{14}O_2$	0.1	-	-	-	-	-	-	-	-	-	-	-
207	Phenanthrene, 1-methyl-7-(1-methylethyl)-	$C1_8H_{18}$	0.3	0.7	0.4	0.4	-	0.6	0.4	0.5	-	-	-	-
209	*n*-Heptadecylbenzene	$C_{23}H_{40}$	-	0.2	0.2	0.2	-	0.4	0.3	0.4	-	-	-	-
211	Abieta-8,11,13-trien-7-one	$C_{20}H_{28}O$	-	0.2	0.2	0.2	-	-	-	-	-	-	-	-
213	Spiro[2,4]heptane, 1,1-diphenyl-5-(diphenylmethylene)-	$C_{32}H_{28}$	-	0.3	-	-	-	-	-	-	-	-	-	-

(continuation)

Peak label	Lignin-derived compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
11	Guaiacol	$C_7H_8O_2$	-	0.2	-	-	-	-	-	-	-	-	-	0.2
57	4-Ethylguaiacol	$C_9H_{12}O_2$	-	-	-	-	0.2	0.2	0.3	0.2	-	-	-	0.3
93	Methoxyethylphenol	$C_9H_{12}O_2$	-	0.2	0.7	0.2	-	-	-	-	-	-	-	-
112	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	-	0.6	1.2	0.8	0.4	0.6	0.7	1.1	1.6	1.5	1.0	1.3
115	Vanillin	$C_6H_8O_3$	-	1.3	0.6	0.8	-	-	0.5	0.6	0.4	0.4	-	0.4
147	Vanillic acid, methyl ester	$C_9H_{10}O_4$	-	0.8	0.7	1.1	-	-	0.4	0.7	-	-	-	-
160	Acetovanillone	$C_9H_{10}O_3$	-	0.4	-	-	-	-	-	0.4	1.3	1.4	1.1	0.8
161	Homovanillic acid	$C_9H_{10}O_4$	-	0.3	-	0.2	-	-	-	-	-	-	-	-
167	4-Allyl-2-methoxyphenol (eugenol)	$C_{11}H_{14}O_2$	-	-	-	-	-	-	-	-	0.8	0.5	0.5	0.6

Peak label	Nitrogenated (N) compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
5	Hydrazine, 1,1- dimethyl	$C_2H_8N_2$	-	1.2	1.3	1.2	0.8	0.7	0.6	0.6	0.6	0.2	0.4	0.4
10	Pyridine	C_5H_5N	-	0.7	0.9	0.8	0.8	0.4	0.4	0.5	0.5	0.4	0.2	0.4
21	1H-Pyrazole.3,5- dimethyl	$C_5H_8N_2$	0.6	-	-	-	-	-	-	-	-	-	-	-
45	Benzonitrile	C_7H_5N	4.0	0.9	0.6	1.3	3.4	1.0	1.1	1.1	-	0.2	0.3	0.6
48	1H-Imidazole-2-carboxaldehyde.1-methyl	$C_5H_6N_2O$	-	-	-	-	-	-	-	-	0.4	0.4	0.3	0.3
85	2-Phenylethanamidine	$C_8H_{10}N_2$	-	-	-	-	-	0.2	0.3	0.4	-	0.3	0.5	0.5
87	3,6-Dimethyl-1H-indazole	$C_9H_{10}N_2$	-	-	-	-	0.3	-	0.2	0.3	-	-	-	-
165	1-H-isoindole- 1,3 (2H)- dione	$C_8H_5N_2$	-	0.8	0.6	0.3	-	-	0.7	-	-	-	-	-
189	Hexadecanenitrile	$C_{16}H_{31}N$	0.4	0.2	0.2	0.2	0.3	0.2	-	-	-	-	-	-

Peak label	Terpenoids	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
18	Camphene	$C_{10}H_{16}$	-	0.2	0.2	0.7	0.5	0.2	0.1	-	0.5	0.4	0.6	0.5
23	β.Phellandrene	$C_{10}H_{16}$	-	-	-	-	-	-	-	-	0.5	0.4	0.5	0.3
24	1,3-Cyclohexadiene. 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	-	0.2	0.2	0.3	-	0.3	0.2	0.2	1.4	1.2	1.8	1.7

(continuation)

Peak label	Terpenoids	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
25	D-Limonene	$C_{10}H_{16}$	-	0.2	0.2	-	-	0.2	0.3	0.3	0.3	0.4	0.4	0.4
26	Sabinene	$C_{10}H_{16}$	-	-	-	-	-	-	-	-	0.7	0.5	0.9	1.0
29	Eucalyptol	$C_{10}H_{18}O$	-	0.5	0.4	0.3	-	-	0.3	0.4	2.0	2.0	2.3	1.7
46	Isoterpinolene	$C_{10}H_{16}$	-	-	-	-	-	-	-	-	-	-	0.3	-
53	verbenene	$C_{10}H_{14}$	-	0.3	-	-	-	-	0.3	-	-	-	-	-
70	Unknown terpenoid	$C_{10}H_{16}O$	-	-	-	-	-	-	-	-	-	0.4	0.4	-
98	Dehydroaromadendrene	$C_{15}H_{22}$	-	-	-	-	-	-	-	-	0.8	0.9	0.7	1.3
103	γ,- Himachalene	$C_{15}H_{24}$	-	-	-	-	-	0.8	0.7	0.6	2.5	2.1	3.4	1.8
104	Dehydroaromadendrene	$C_{15}H_{22}$	-	-	-	-	-	-	-	-	0.4	0.4	0.7	0.6
109	Alloaromadendrene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	0.4	0.4	0.5	0.5
110	Alloaromadendrene	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	0.9	0.5	0.3	1.3
114	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	0.6	0.6	1.0	0.6
116	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	3.6	3.1	4.8	2.9
117	Unknown terpenoid	$C_{15}H_{24}$	-	-	0.7	0.4	-	-	-	-	-	-	-	-
124	Alloaromadendrene	$C_{15}H_{24}$	-	0.1	0.2	-	-	0.3	0.4	0.2	0.5	0.5	0.4	0.6
126	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	1.2	1.2	1.1	1.4
131	Dehydroaromadendrene	$C_{15}H_{24}$	-	-	-	-	-	-	0.3	0.2	0.5	0.6	0.4	0.3
132	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	0.7	0.6	0.7	0.9
133	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	1.4	1.3	2.6	1.9
134	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	0.2	0.5	-	0.3	0.2	1.8	1.8	3.7	1.4
136	Unknown terpenoid	$C_{15}H_{24}$	-	-	-	-	-	-	-	-	0.8	0.7	0.7	0.8
137	Dehydroaromadendrene	$C_{15}H_{22}$	-	-	-	-	-	-	-	-	0.6	0.4	-	0.9
138	α-Gurjunene	$C_{15}H_{24}$	-	0.4	0.5	0.4	-	0.4	0.2	0.1	-	-	-	-
139	Dehydroaromadendrene	$C_{15}H_{22}$	0.2	0.5	0.3	0.3	-	-	-	-	0.8	0.3	0.5	0.4
145	α-calacorene	$C_{15}H_{20}$	-	0.2	-	0.4	-	-	-	-	0.5	0.3	0.4	0.3
148	α- calacorene	$C_{15}H_{20}$	-	0.1	-	0.2	-	-	-	-	0.4	0.4	0.4	0.9
149	Globulol	$C_{15}H_{26}O$	-	-	-	-	-	-	0.5	0.5	0.5	0.6	0.5	1.0
156	Dehydroaromadendrene	$C_{15}H_{22}$	-	-	-	-	-	-	-	-	0.6	0.7	0.5	0.3

(continuation)

Peak label	Hydroaromatic steroids	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
227	Unknown steroid	$C_{27}H_{54}O$	-	-	-	-	-	-	-	-	0.1	-	-	0.3
228	Unknown steroid	(blank)	-	-	0.1	0.2	-	-	-	-	0.2	0.3	0.2	0.2
229	Unknown steroid	(blank)	-	0.2	0.2	0.2	0.3	0.2	0.2	-	0.2	0.3	0.3	0.2
230	β-Sitosterol (Stigmast-5-en-3b-ol)	$C_{29}H_{50}O$	-	0.1	0.09	0.1	0.2	0.3	0.3	0.3	0.1	-	0.2	-
231	Unknown steroid	(blank)	-	-	0.1	0.1	0.08	0.2	0.2	0.2	-	0.09	0.2	0.3
232	Unknown steroid	(blank)	-	0.07	0.08	0.06	-	-	0.1	0.2	0.1	0.2	0.2	0.3
233	Unknown steroid	(blank)	-	-	0.2	0.2	0.2	0.3	0.3	-	0.2	0.4	0.3	-
234	Preussomerin I	$C_{21}H_{14}O_8$	-	-	-	-	-	-	-	-	0.2	0.3	-	-
235	β Amyrin	$C_{30}H_{50}O$	-	-	-	-	-	0.2	0.2	0.2	-	-	-	-
236	Stigmastan-3,5-diene	$C_{29}H_{48}$	-	0.2	0.2	0.2	-	-	0.1	0.2	0.4	0.7	0.3	0.5

Peak label	Polysaccharides– derived compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
2	Furan, 3-methyl-	C_5H_6O	2.0	0.7	0.8	0.6	0.9	0.9	0.8	0.7	0.5	0.5	0.4	0.9
6	2-Vinylfuran	C_6H_6O	-	-	-	-	-	0.2	0.3	0.3	-	-	-	-
17	Furfural	$C_5H_4O_2$	-	1.5	1.5	1.6	-	0.7	1.1	1.0	2.3	2.1	2.2	2.8
22	2-Cyclopenten-1-one, 3-methyl-	C_6H_8O	-	0.9	0.8	-	1.1	1.1	1.2	0.6	0.5	0.4	0.1	0.5
32	Benzofuran	C_8H_6O	-	-	1.4	0.8	-	-	1.7	2.0	-	-	-	-
34	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	-	-	-	1.9	-	-	-	-	1.5	1.6	1.1	1.8
35	Benzofuran	C_8H_6O	0.2	-	-	-	-	-	-	-	-	-	-	-
38	2-Furancarboxaldehyde, 5-methyl-	$C_6H_6O_2$	-	1.6	1.9	1.0	4.0	2.3	2.2	2.5	-	-	-	-
43	2-Cyclopenten-1-one, 3-methyl-	C_6H_8O	-	0.3	0.5	-	-	0.4	0.1	0.3	-	-	-	-
51	1,6- Anhydro-beta-D-glucopyranone (levoglucosan)	$C_6H_{10}O_5$	-	-	-	0.3	-	-	-	-	-	-	-	-
56	2-cyclopenten-1-one.2-hydroxy- 3-methyl	$C_6H_6O_2$	-	-	-	-	-	-	-	-	0.5	0.4	0.3	0.6
61	Benzofuran, 2-methyl-	C_9H_8O	-	0.1	0.4	0.4	-	-	-	-	-	-	-	-
78	Furan, 2-(1-pentenyl)-. (E)-	$C_9H_{12}O$	-	1.2	-	-	-	-	-	-	-	-	-	-
81	2,3- dihydro-benzofuran	C_8H_8O	-	0.8	0.9	1.2	0.6	1.1	1.9	2.5	-	-	-	-

(continuation)

Peak label	Polysaccharides– derived compounds	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
113	3-Benzofurancarboxylic acid. 2,3-dihydro-	$C_9H_8O_3$	-	-	-	-	-	-	-	-	-	-	-	-
121	1H-Inden-1-one. 2,3-dihydrotetramethyl-	$C_{13}H_{16}O$	0.3	-	-	-	-	-	-	-	-	-	-	-
129	1H-Inden-1-one. 2,3-dihydro-3,3,5,7-tetramethyl-	$C_{13}H_{16}O$	0.1	0.5	0.3	0.3	0.3	0.4	0.4	0.5	0.4	0.3	0.4	0.2
140	d-allose	$C_6H_{12}O_6$	-	-	1.7	1.9	-	-	-	-	2.3	1.4	2.1	0.8

Peak label	Other compounds (unspecific)	Molecular formula	Avg. %TIC[a]											
			Sediment (SF–EB)				Burnt soil (EB)				Unburnt soil (EU)			
			I	II	III	IV	I	II	III	IV	I	II	III	IV
1	3-Penten-1-ol	$C_5H_{10}O$	8.0	6.0	6.0	4.0	6.0	4.0	3.0	4.0	1.0	1.0	2.0	1.0
3	1,5-Pentanediol, 3-methyl-	$C_6H_{14}O_2$	-	0.9	0.7	0.4	-	-	0.8	0.7	-	-	-	-
7	2,4-Heptadien-1-ol	$C_7H_{12}O$	-	-	-	-	-	0.3	0.4	0.2	0.4	0.5	-	-
39	cyclohexanepropanal	$C_9H_{16}O$	-	-	-	0.7	-	-	-	-	-	-	-	-
41	2,6- dimethyl-7-octen-1-ol	$C_{10}H_{20}O$	-	0.2	0.4	-	-	-	-	-	-	-	-	-
49	Bicyclo [4.1.0]heptan-2-one, 3,7,7-trimethyl-	$C_{10}H_{16}O$	-	0.2	0.2	0.2	-	-	0.3	0.6	-	-	-	-
55	2-Nonanone	$C_9H_{18}O$	-	0.4	0.6	0.5	0.8	-	0.6	0.5	-	-	-	-
96	2-Undecanone	$C_{11}H_{22}O$	0.2	0.3	0.2	0.3	0.5	0.3	-	-	-	-	-	-
183	2-Heptadecanone	$C_{17}H_{34}O$	-	-	0.2	0.3	0.2	0.4	0.3	0.5	0.2	0.3	0.3	0.2
188	2-Pentanone. 1- (2,4,6- trihydroxphenyl)	$C_{11}H_{14}O_2$	-	-	-	-	-	-	-	-	0.3	0.3	0.2	0.2
208	1-Eicosanol	$C_{20}H_{42}O$	-	-	-	-	-	-	-	-	0.2	0.3	0.2	-

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2.4. Post-fire dissolved and particulate organic carbon export by overland flow in two Mediterranean eucalypt stands (article IV)

This chapter (in preparation):

***Faria, S.R.**,* Varela, M.E., Esteves, V.I., *Keizer, J.J. 2015. Post-fire dissolved and particulate organic carbon export by overland flow in two Mediterranean eucalypt stands.*

Abstract

Although numerous studies have documented the effects of wildfires on hydrological and erosion processes, the associated transport of organic carbon (OC) has received considerably less research attention. The present study estimates post-fire OC exports by overland flow from two eucalypt plantations during the first year following the wildfire at the micro-plot scale (0.25 m^2). The temporal patterns of these OC exports were also analysed. Total organic carbon (TOC) content in runoff samples was measured with a Shimazdu TOC-analyser at 1- to 2- weekly intervals. The main findings of this study were the following: (i) the amount of deposited ashes was higher at the NW slope than at the SE slope, while ashes total organic carbon content revealed no differences; (ii) total sediment losses and also the total soil organic carbon export were higher at the NW slope than at the SE slope; (iii) particulate organic carbon fraction showed the highest loss at the both topsoil sites. In addition, the present study provides some insight into post-fire organic carbon losses in the recently burnt areas, which is crucial information for ecosystem management.

Introduction

Wildfire is currently a dominant factor and matter of concern in many forests ecosystems in across the world (Shakesby and Doerr, 2006). In Portugal, like in other Mediterranean countries, wildfire frequency and intensity have intensified over the last decades, as result of socio-economic changes and in particular, land abandonment and afforestation with fire-prone tree species such as pine and eucalypt, as well as of an increase in meteorological conditions propitious to wildfires (Pereira et al., 2006; Marques et al., 2011; Shakesby, 2011).

Soil is an important ecosystem component that can be extensively modified as a result of wildfire, either directly or indirectly. Besides partially combusting litter layer, wildfires can lead to considerable topsoil heating and thereby, lead to marked changes in the physical, chemical and biological properties of the topsoil (DeBano et al., 1998; Shakesby and Doerr, 2006). In turn, fire-induced changes in physical properties, such as infiltration capacity, soil water repellency and aggregate stability, can substantially increase runoff generation and the associated soil (fertility) losses during the so-called windows-of-disturbance (Shakesby and Doerr, 2006; Mataix-Solera et al., 2011).

Considerable post-wildfire erosion rates has been observed in burnt Mediterranean forest. Shakesby (2011) reported soil erosion rates ranged between 0.10− 0.39 Mg ha^{-1} y^{-1} for low severity fire, 2.10−3.28 for moderate severity and 3.70−10.8 Mg ha^{-1} y^{-1} for high severity measured on field plots in the Mediterranean areas one year after the fire. Other studies in north-central Portugal reported soil losses of 0.6 Mg ha^{-1} y^{-1} during the first two post-fire years in a Pine plantation (Martins et al., 2013) and 1.1−8 Mg ha^{-1} y^{-1} in eucalypt plantations during the first year after fire (Prats et al., 2014; Faria et al., 2015).

The loss of soils through erosion and overland flow can represent an important pathway of soil organic carbon (SOC) loss, either in dissolved or in particulate form (DOC and POC). However, the post-fire SOC impacts have been poorly studied in the Mediterranean region and, in particular in Portugal. Shakesby et al. (2013) reported modest fire effects on soil quality, since the total estimated post-prescribed fire particulate losses of OM represented only 0.5–2.9% of the content in the upper 2 cm of soil. Therefore, there is a need to investigate the post-fire effects on SOC export behavior.

Dissolved organic carbon (DOC), a relatively mobile fraction and one of the most active of the SOC, plays an important role in many biogeochemical processes and in the C cycle in the forest ecosystems (Kalbitz et al., 2000; Jardine et al., 2006; Bolan et al., 2011). It also forms a major pathway for C transfer from terrestrial to aquatic ecosystems and facilitates the transport and/or bioavailability of nutrients and pollutants, such as nitrogen (N), phosphorus, sulphur and trace metals (Qualls and Haines, 1991; Kalbitz et al., 2000). The main source of DOC are a number of biologically and/or biochemically mediated processes, including plant and root litter decomposition and leaching, SOM mineralization, root exudation, mucilage and microbial activity (Kalbitz et al., 2000; Hansson et al., 2010). However, the concentration of DOC in soils is controlled not only by its production, but also by its consumption through organisms, hydrologically driven transport and physico-chemical retention (Dalva and Moore, 1991; Neff and Asner, 2001; Kalbitz et al., 2003).

Generally, the DOC input from forest ecosystems have been reported in a range of 1 g m^{-2} y^{-1} and the export ranged between 10-50 g m^{-2} y^{-1} in the O horizon and below 10 g m^{-2} y^{-1} in the B horizon (Michalzik et al., 2001; Moore, 2003). However, studies in wildfire effects and export of DOC and particulate organic carbon (POC) in forest areas also have been very limited. In this way, the main aim of this study was to provide initial estimates of possible carbon exports by overland flow from recently burnt eucalypt plantations. More specifically, this study wanted: (i) to compare, for two adjacent eucalypt plantations, the carbon export by the runoff events during the first year following the wildfire; (ii) to compare the carbon losses at the scale of micro-plot (iii) to analyse the temporal patterns in carbon losses; (iv) to determine the contributions of the dissolved versus particulate fraction and, in the case of the dissolved fraction, of organic versus inorganic carbon.

Materials and Methods

Caracterization of the study sites

The study was conducted in north-central Portugal, located near the Ermida village (40° 08' 46" N; 7° 59' 35" W; 500 m a.s.l.) in the Sever do Vouga municipality, Aveiro District. In the burnt area, 300 ha of forest lands, predominantly covered by eucalypt plantations (*Eucalyptus globulus* Labill.) were burned by a wildfire at the end of July 2010. Overall, the fire severity of this wildfire was moderate, according to the methodology described in Shakesby and Doerr (2006) and Keeley (2009), and as detailed in Faria et al. (2015) and Machado et al. (2015).

The study area has a humid meso-thermal Mediterranean climate (Csb in the Köppen classification), with moderately dry but extended summers (DRA-Centro, 1998). Mean annual temperature at the nearest weather station at approximately 13 km to the North of the study area (Castelo Burgães: 40º 51'16"N, 8º 22'55"W; 306 m a.s.l) was 14.9 ºC (SNIRH, 2011 :1991-2011). Long-term annual rainfall at the nearest rainfall station at approximately 4.5 km to the east of the study area (Ribeiradio: 40º 44'39"N, 8º18'05"W; 228m a.s.l) was, on average, 1609 mm, but varied markedly between 960 and 2530 mm (SNIRH, 2011 :1991-2011). The study area pertains to the physiographic unit of the Hespheric Massif, which is mainly composed of pre-Ordovician schists and graywackes, while Hercynian granites occur at specific locations (Ferreira de Brum, 1978).

Within the burnt area, two hillslopes were selected for the present study (Table 12). The two sites were explicitly chosen for sharing the same forest type (eucalypt plantation), the same fire severity (moderate), the same parent material (schist) and the same steepness (19-20º) and while contrasting in exposition. The EB-S1 site had an SE exposition, while the EB-S2 site had a NW exposition (Table 12).

Table 12 - General description of the two study sites.

Site	SE	NW
	EB-S1	EB-S2
General characteristics		
Forest type	*Eucalyptus globulus* Labill.	
Bedrock	Schist	
Elevation	150	266
Orientation	SE	NW
Slope angle (°)	20.0 ± 2.0	19.0 ± 3.0
Slope length (m)	60	90
Coordinates	40°43'30'' N; 8°20' 57''W	40°44'5'' N; 8°21 15''W
Fire severity indicators		
Consumption of tree crowns	Partial	Partial
Consumption of shrub layer	Total	Total
Consumption of herbs/litter	Total	Total
Ash colour	Black	Black
Soil characteristics		
Soil type (IUSS, 2014)	Umbric Cambic Leptosols (loamic)	
0-2 cm		
Texture class	sandy-clay loam	sandy loam
Sand fraction (%)	67	71
Silt fraction (%)	9	10
Clay fraction (%)	24	18
OM (%)	28 ± 4	32 ± 12
Stone content (>2 mm; % w/w)	64	41
0-5 cm		
Bulk density ($g.cm^{-3}$)	1.15 ± 0.19	0.88 ± 0.13

The soils at the two study sites were described in the field and classified as Umbric Cambic Leptosols (Loamic), having shallow A-horizons of 13−20 cm overlying C− or R− horizons consisting of schists (IUSS, 2014). During the description of soil profiles, soil (0-2 cm depth) samples were collected at the bottom of the slope. These samples were analyzed in the laboratory to determine bulk density (Porta et al., 2003) and soil texture (Guitián and Carballas, 1976). The texture of the soils at the study sites varying between sandy-clay loam and sandy loam (Table 12).

Experimental design and field sampling

The two study sites were instrumented with four micro-plots, divided over two pairs that were placed at the base and halfway the slope section, while the plots of each pair were placed at 2-3 m distance from each other. The micro-plots were roughly 0.5m by 0.5m and had a downslope triangular outlet area of roughly 0.03 m^2, metal sheets of 15 cm high that were driven some 5 cm into the soil. The outlets were connected to garden hose that directed the runoff into, one or more high-density polyethylene tanks of 30 L to collect the overland flow. The installation of the plots was finalized by 31 August 2010, before the first rainfall event after the wildfire.

From 25 August 2010 until 9 October 2011, the runoff collected in the tanks was measured at 1- to 2- weekly intervals, mainly depending on the occurrence of rainfall, at a total of 45 occasions (i.e. read-outs). Whenever the runoff in a tank exceeded 250 mL, two samples were collected following thorough stirring of the water. One sample was collected in a 500 mL polyethylene bottle that had been previously rinsed with hydrochloric acid (pH < 2.0) and distilled and deionized water, while the other sample was collected in a 1.5 L bottle. The former samples were stored in cool boxes during the field work and, upon arrival in the laboratory, stored in a fridge at 4°C for no longer than 24 h.

At both study sites, the ash layer and the topsoil (0–2 cm depth) were sampled on 30 August 2010, also before the first rainfall event after the wildfire. To this end, a transept was laid out at each site from the base to the top of the slope, along which five equidistant points were established. At each transect point, first all ashes were carefully collected over an area of 0.25 m^2 (0.5m × 0.5 m), after which the same was done for the upper 2cm of the mineral soil using a small branch over an area of 0.06 m^2 (0.25 m × 0.25 m). Upon arrival in the laboratory, the ash and topsoil samples were air-dried at room temperature, then sieved to the fine-earth fraction (> 2 mm), and finally stored under dark and cool conditions until further analysis.

Laboratory analysis

Within 24 h upon arrival in the laboratory, the runoff samples collected in a 500 mL polyethylene bottle were filtered through glass fiber filter (0.45 µm; Whatman grade GF/F), after which both filter and filtrate were stored at -18° C until further analysis. The contents of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) in the filtrate and of particulate organic carbon (POC) of the filter were measured using a Total Organic Carbon Analyzer (ShimadzuTOC−5050A) and a coupled SSM-5000 module (Shimadzu Corp. Japan), respectively. The total organic carbon (TOC) content of the runoff samples was then calculated as the sum of the DOC and POC fractions.

The TOC content of the ash and topsoil samples was also measured using the above-mentioned SSM module. All analyses with the TOC Analyzer and its SSM module done with an analytical precision of 0.2%, so that, the average of 3 readings per sample was considered to be representative.

The samples' sediment concentration (in a 1.5 L bottle) was determined through filtration over a paper filter with a pore diameter of 12-15 µm, followed by drying at 105 °C for 24h (APHA, 1998) and for their organic concentration (Botelho da Costa, 2004).

Data analyses

The DOC, DIC, POC and combined exports at the different sampling occasions were related to selected potential explanatory variables by means of the Spearman correlation coefficient (Zar, 1999). These variables were rainfall amount and intensity, overland flow volume, sediment losses, following (Machado et al., 2015). Spearman correlation coefficients were performed using SigmaPlot 11.0 package software, and tested for significance using an α of 0.05.

Results and discussion

Ash amounts and their organic carbon contents and stocks

At the first sampling occasion in August 2010, immediately after the wildfire, the amount of deposited ashes was roughly 2.5 times higher at the NW slope than at the SE slope, with average values of 206 $g.m^{-2}$ (EB-S2) and 76 $g.m^{-2}$ (EB-S1) (Table 13). These contrasting results probably reflected differences in pre-fire loads, especially since fire severity was classified as moderate at both sites, on the one hand, and, on the other, since biomass properties (type, weight, moisture) together with fire characteristics (intensity, duration, micrometeorological conditions) are considered the key factors determining ash quantity (Neary et al., 2005; Bodí et al., 2014).

Few studies, however, have quantified the amounts of ashes after a wildfire. For example, for conifer forest in USA and Spain, values ranging from 1500 – 15120 $g.m^{-2}$ have been reported (Goforth et al., 2005; Cerdà and Doerr, 2008; Woods and Balfour, 2008). One reason for this knowledge gap may be, presumably, rapid changes in ash amounts following wildfire, especially through wind and water erosion (Bodí et al., 2014). For an eucalypt forest in Australia, Santín et al. (2012) reported a mean ash load that was roughly ten times higher than that at the EB-S1 site (i.e 8187 $g.m^{-2}$). This discrepancy could be due to the difference in the nature of the studied eucalypt stands (managed vs. spontaneous) and associated pre-fire biomass well as to the difference in the severity of the wildfire (moderate vs. severe) and thus, the consumption of the pre-fire biomass. Along these same lines, a recent study carried out by Santín et al. (2015) in an Australia eucalypt forest revealed that ash loads were positively related to fire severity (as inferred from the degree of vegetation and ground fuel destruction).

The ashes of the two sites differed little in average total organic carbon (TOC) content, being slightly highest at the NW slope (EB-S1: 31%) than at the SE slope (EB-S2: 35%). These figures were 4-5 times higher than the ash TOC content reported by Santin et al. (2012) for an eucalypt forest in Australia.

Table 13 - Average of ash and topsoil (in)organic carbon (TOC;TIC) contents and stocks as well as rainfall and average of overland flow volumes, runoff coefficients, sediment losses and (in)organic dissolved (DIC; DOC) and particulate carbon (POC) losses at the micro-plot scale at the two eucalypt plantations over the first year after a wildfire.

	SE	NW
	EB-S1	EB-S2
Hydrometeorological parameters		
Rainfall (mm)	1472	
Overland flow (mm)	407 ± 204	345 ± 187
Runoff coeficient (%)	28 ± 17	23 ± 15
Sediment losses ($g.m^{-2}$)	140 ± 123	415 ± 222
Carbon contents and stocks		
Ash		
Amounts ($g.m^{-2}$)	76 ± 9	206 ± 13
TOC content (%)	31 ± 4	35 ± 7
TOC stock [a] ($gC.m^{-2}$)	23 ± 11	71 ± 33
TIC content (%)	n.d [c]	n.d [c]
Topsoil (0-2 cm depth)		
TOC content (%)	15 ± 0.2	18 ± 7
TOC stock [b] ($gC.m^{-2}$)	1259 ± 17	1855 ± 711
TIC content (%)	n.d [c]	n.d [c]
Total (In) Organic carbon exports in overland flow		
POC losses ($g.m^{-2}$)	23 ± 1	66 ± 3
DOC losses ($g.m^{-2}$)	3.8 ± 0.2	3.9 ± 0.1
DIC losses ($g.m^{-2}$)	0.6 ± 0.02	3.0 ± 0.2
TOC losses ($g.m^{-2}$)	27 ± 1	70 ± 3

[a] Ashes total organic carbon stock is estimated from: $OC \times Q \times d$; *w*here *OC* is the ashes/soil organic carbon content; *Q* is amount of ashes/soil over an area of 1 m^2, *d* is depth of ashes layer

[b] Soil organic carbon stock is estimated from: $OC \times Q \times d \times (1-G)$; *G* is the content of the > 2 mm fraction

[c] Total inorganic carbon in ashes/topsoil was below the detection limit of the Total Organic Carbon Analyser

The greater OC content of the ash may be due to higher proportion of pyrogenic organic material, i.e charcoal-type C enriched materials. Faria et al. (2015) have reported a relative abundance of almost 80% of thermally-altered compounds, such as aromatic non-specific compounds, nitrogenated compounds and polysaccharide-derived compounds in eroded sediments (constituted essentially by ashes) after one month after wildfire in the study area. Furthermore, the present TOC contents were at the higher end of the range reported by the studies on wildfire ashes in Mediterranean forests reviewed by Bodí et al. (2014: 4.6 - 31.1%), as well as of the range found by Badía-Villas et al. (2014: 7.1 - 42.1%).

The average TOC stock of the ash layer was thrice as high at the NW site (EB-S1: 71 $g.m^{-2}$) than at the SE slope (EB-S2: 23 $g.m^{-2}$), mainly reflecting differences in ash amounts. Even the highest figure, however, was lower than the ash TOC stocks reported by Santín et al. (2012: 593 $g.m^{-2}$ and by Santín et al. (2015: 96 $g.m^{-2}$ for an eucalypt forest in Australia. As detailed above, this discrepancy was mainly due to differences in ash amounts, compensating the differences in ash TOC contents.

Topsoil organic carbon contents and stocks

As the TOC content of the ashes, the TOC content of the topsoil (SOC) immediately after the wildfire did not differ much between the two study sites but was somewhat higher at the NW slope than at the SE slope (Table 2: 18 vs. 15 %). Both values were markedly higher than the TOC content of 3.9 % reported for another recently burnt eucalypt stand in north-central Portugal (De la Rosa et al., 2012), which could be explained by the different sampling depth (0-5 cm) and/or by the different timing of the sampling (10 months after the wildfire). This role of time-since-fire was suggested by the finding of Campo et al. (2008) for post-fire soil organic matter (SOM), which the authors attributed in part to seasonal changes and especially to drought occurrence, in accordance with the findings of Faria et al. (2015) for burnt eucalypt plantation immediately outside the burnt area studied here. The present topsoil TOC contents were also higher than values reported by other studies in Mediterranean region. Badía-Villas et al. (2014) found SOC contents of 8.3% at 0-2 cm of soil depth whereas de Blas et al. (2013) reported values ranged between 8.0-13.6% at 0-5 cm of soil depth for eucalypt forest. In contrast, however, the SOC contents was 2-3 times lower than for eucalypt forest in Australia at 3-8 cm of soil depth

(Santín et al., 2015). The SOC stock immediately after the fire was, on average, 1.5 times larger at the NW site than at the SE site (Table 2: 1855 vs. 1259 $g.m^{-2}$). This difference was to large extent due to the lower stone content of the topsoil at the NW slope, also compensating its lower dry bulk density (see Table 13).

In Portugal, the only relevant study known to the authors was carried out by Rosário (2010) who reported similar SOC stocks, ranging between 1250-1500 $g.m^{-2}$ (0-5 cm of soil depth) for unburnt eucalypt plantation. In case of Mediterranean burnt areas, Certini et al. (2011) also have reported similar values, with SOC stocks of 1660 $g.m^{-2}$ (0-5 cm), eleven months after fire in pine forest.

Overall rainfall and runoff amounts and sediment and organic carbon losses by overland flow

The rainfall during the study period from 25 August 2010 to 9 October 2011 amounted to 1472 mm and, thus, closely approximated the mean annual rainfall at the closest rainfall station with long-term records (Ribeiradio: 1609 mm). This rainfall produced, on average, some 18% more overland flow at the SE site than at the NW site (Table 13: 407 *vs.* 345 mm). This runoff difference could be related to the higher stone cover at the SE site than at the NW site, amounting to 30-40 % as opposed to <10 % from winter 2010 onwards, especially by decreasing the infiltrating area. The corresponding runoff coefficients at the two study sites of 28 and 23 % were 2-2.5 times lower than the average value of 55% reported by Prats et al. (2014) for a SW-facing eucalypt stand located opposite to EB-S2 over basically the same monitoring period and using the same micro-plots. They were, however, comparable to the average micro-plot-scale runoff coefficients of two other eucalypt plantations in the present study area, a SE-facing one on schist and an E-facing one on granite (Machado et al., 2015: 33 and 25 %, respectively), albeit this latter study was limited to the first 7 months after the wildfire. The present figures also agreed well with the average micro-plot-scale value of 22 % that was observed at both of two adjacent eucalypt plantations on schist in the study region over the first post-fire year, notwithstanding their contrasting expositions and pre-fire land management practices

(Malvar et al., 2015: SE vs NE; ploughed vs. unploughed). In contrast, to runoff volumes, total sediment losses over the entire study period were, on average, clearly greater (approximately 3 times) at the NW slope that at the SE slope (Table 13: 415 *vs.* 140g m^{-2}). This inverted between-site difference reflected distinct specific sediment losses, averaging 1.2 g m^{-2} mm^{-1} runoff in the case of the NW slope as opposed to 0.3 g m^{-2} mm^{-1} runoff in the case of the SE slope. Likewise, Keizer et al. (2015) found that the median sediment concentration of micro-plot-scale runoff samples was notably higher at the NW slope than at the other three eucalypt plantations on schist studied in this same area, including the present SE slope (0.73 vs. 0.21-0.41 g L^{-1}). The smaller sediment losses at the NW than SE slope could be explained by the higher cover of litter and especially stones, amounting to 40-70 % as opposed to <10-30 % throughout the study period (Figure 21).

This higher cover, could have increased protection against splash erosion and enhanced resistance to flow, thereby reducing the detachment and transport capacity of the overland flow (Shakesby, 2011). In any case, at both sites the average annual sediment losses over the first post-fire year exceeded the 1 Mg ha^{-1} y^{-1} threshold for tolerable soil loss proposed by Verheijen et al. (2009; 2012). Compared to micro-plot-scale sediment losses reported for other eucalypt plantations in the study region over the first post-fire year, the present figures were at least four times smaller than the average value of 1730 g m^{-2} y^{-1} reported by Prats et al. (2014) but agreed well with the average values of 378 and 112 g m^{-2} y^{-1} found by Malvar et al. (2015) at the unploughed and ploughed site, respectively. Unlike was the case here, however, the between-site discrepancy in erosion rates in Malvar et al. (2015) seemed unrelated to differences in protective soil cover by vegetation, litter and stones.

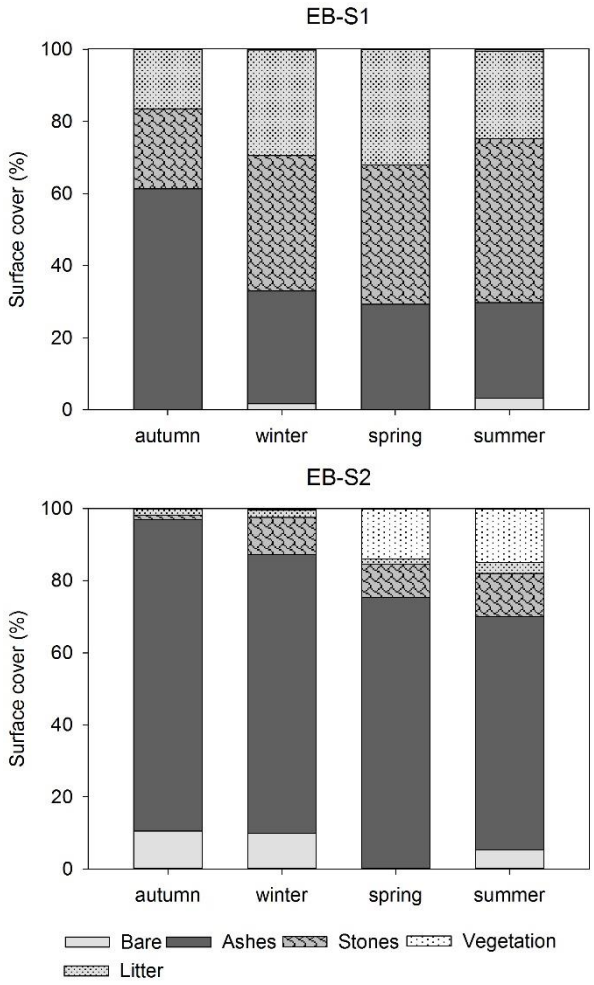

Figure 21 – Mean surface cover (%) by period after the fire in the two sites.

In close agreement with the overall sediment losses, TOC losses by overland flow were, on average, 2.7 times greater at the NW slope than at the SE slope. They amounted to 70 and 27 g C m^{-2} (Table 2), and thus correspond to 17 and 19 % of the sites' overall sediment losses, respectively. Prior micro-plot-scale studies in recently burnt eucalypt stands in the study region have found that SOM (determined by loss-on-ignition) constituted a markedly larger fraction of the sediments eroded during the first post-fire year, ranging from 29 to 47 % under simulated rainfall conditions (Malvar et al., 2011; 2013) and from 50 % (Malvar et al., 2015) to 61 % (Prats et al., 2014) under natural rainfall conditions.

Interestingly, the TOC exports were very similar to the TOC stocks of the ashes at the respective sites immediately after the wildfire (Table 2: 71 and 23 g C m^{-2}). Nonetheless, ash cover was still quite substantial towards the end of this study, during the summer of

2011, especially at the NW site (approximately 60%) where TOC losses were greatest. The TOC losses furthermore corresponded to 3.7 and 2.2% of the combined TOC stocks of the ash layer and the upper 2 cm of the topsoil at the NW and SE site, respectively. These figures compared reasonably well with those in Shakesby et al. (2013) with respect to SOM losses by overland flow over the first year after a prescribed fire in a shrubland in north-central Portugal. It must be noted, however, that the authors did not include the SOM stock of the ash layer.

TOC losses at both study sites were predominantly in particulate form (POC), corresponding to 90% in the case of the NW site and to 85% in the case of the SE site. The absolute losses in dissolved inorganic carbon (DIC) revealed the same pattern as the TOC and POC losses, being 5 times greater at the NW site than the SE site (3.0 and 0.6 gC m^{-2}). In contrast, the losses in dissolved organic carbon (DOC) were basically the same for the two study sites (3.8 and 3.9 gC m^{-2}). This discrepancy between DIC and DOC losses could be explained by different dissolution and/or transport processes at the two sites, or by distinct enrichment factors of DIC in the sites' ashes. The latter would seem most likely, as DIC fractions have been found to depend on thermal decomposition reactions during a fire (Gu et al., 2008). Although fire severity at both sites was classified as moderate, the DIC results could be related to different burning conditions, leading to greater enrichment of inorganic constituents in the ashes at the NW site. For example, Goforth et al. (2005) reported carbonates and bicarbonates as substantial constituents in ash, accounted for up to 10% of sample weight for 'white ash' from a severely burnt mixed-species conifer forest.

Seasonal patterns in POC, DOC and DIC exports by overland flow

The exports of dissolved and particulate organic carbon (DOC and POC) as well as dissolved inorganic carbon (DIC) of the individual plots are summarized for the different seasons of the first post-fire year in Figure 22. All three fractions revealed larger losses over autumn 2010 than over the three subsequent seasons at both study sites. The fractions' temporal patterns in carbon exports differed in that: (i) POC losses were higher over winter 2010/11 than over spring and summer 2011, whilst DOC and DIC losses were not; (ii) DOC losses were higher over summer 2011 than over winter 2010/11 and spring

2011, whilst POC and DIC losses were not. These different temporal export patterns sustained the earlier inference that the three fractions appeared to involve distinct detachment/dissolution and/or transport processes. The gradual decrease in POC exports agreed well with the well-established classical model of gradually decreasing post-fire sediment losses with increasing time-since-fire (Shakesby and Doerr, 2006; Shakesby, 2011), and could be explained by a gradual exhaustion of the ash layer. The validity of this model for eucalypt plantations in the study region was suggested by prior micro-plot scale studies, observing larger sediment losses during the first few months after the fire than during the remaining months of the first post-fire year (Prats et al., 2014; Malvar et al., 2015). However, Prats et al. (2014) and Malvar et al. (2015) also found peaks in sediment losses during the autumn months at the start of the second year after the fire, in line with the increase in DOC export from spring to summer 2011 reported here. In contrast, the present DIC losses suggested a much quicker exhaustion of DIC than of DOC or, alternatively, a rapid immobilization (Gu et al., 2008). The above-mentioned differences in overall POC losses between the two study sites were mainly due to higher average exports at the NW than SE site over autumn 2010 and winter 2010/11. In the case of DIC, the overall difference reflected larger average losses during the first post-fire season.

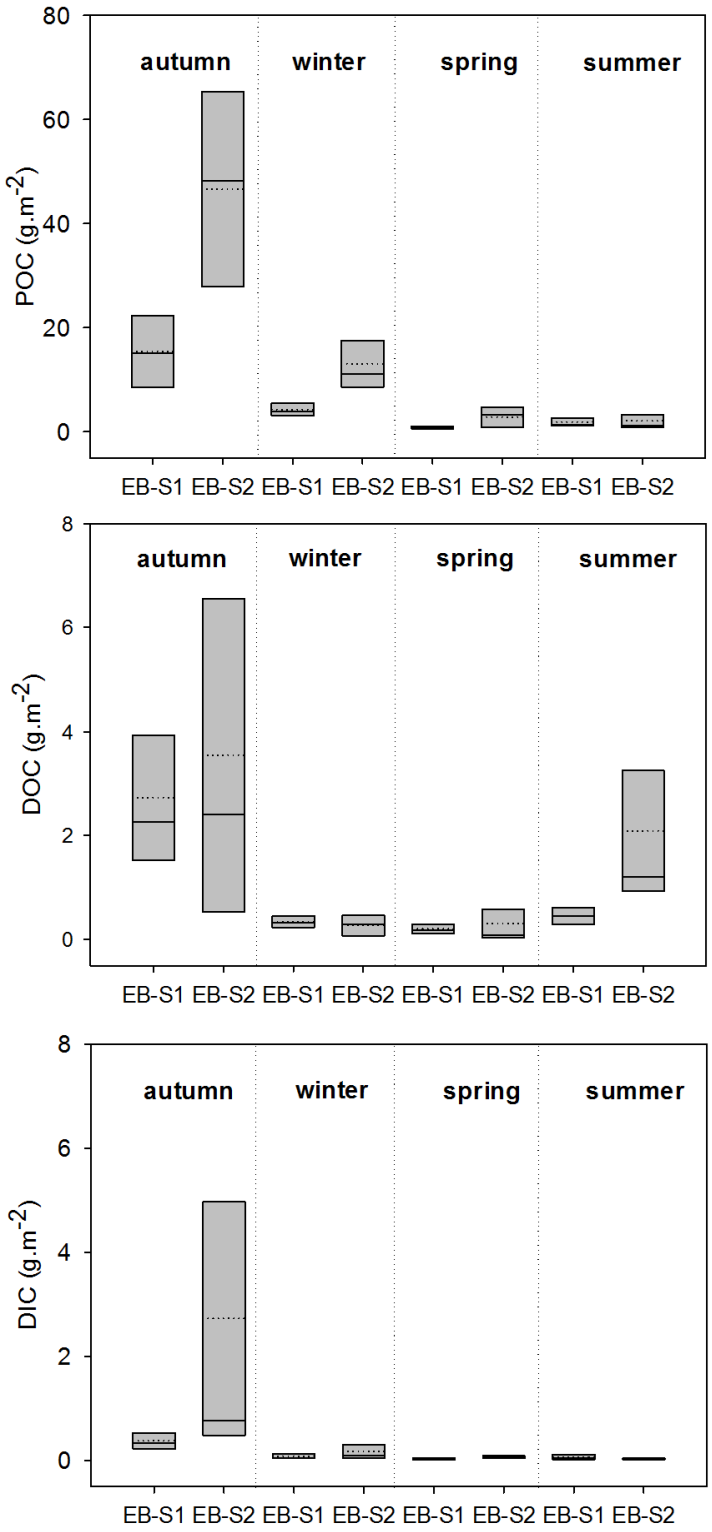

Figure 22 - Dissolved organic carbon (DOC), particulate organic carbon (POC) and dissolved inorganic carbon (DIC) export at two burnt eucalypt sites.

The average losses of all three fractions were significantly and strongly associated with rainfall amounts, overland flow volumes and sediment losses of the individual measurement periods at both study sites (Table 14). At both sites, the Spearman rank correlation coefficients were highest for overland flow volumes (0.85-0.96), intermediate for rainfall amounts (0.78-0.91) and lowest for sediment losses (0.67-0.86). This was true for DOC, DIC and, perhaps somewhat surprisingly, also POC.

Table 14 - Spearman Rank correlations of organic carbon losses (averages per read out) with hydro-meteorological (rainfall amount, overland flow volumes and sediment losses). Values significantly different from zero at $\alpha \leq 0.05$ are present in bold.

Slope	Variable	DOC	POC	DIC
EB-S1	Rainfall amount (mm)	**0.87**	**0.91**	**0.89**
	Overland flow volume (mm)	**0.93**	**0.96**	**0.94**
	Sediment losses ($g.m^{-2}$)	**0.72**	**0.76**	**0.73**
EB-S2	Rainfall amount (mm)	**0.78**	**0.91**	**0.90**
	Overland flow volume (mm)	**0.85**	**0.92**	**0.94**
	Sediment losses ($g.m^{-2}$)	**0.67**	**0.86**	**0.76**

Likewise, micro-pot scale cation (Na^+, K^+, Ca^{2+}) exports in two other eucalypt stands in the same burnt area as studied here tended to be better correlated with overland flow volumes than with rainfall amounts and especially sediment losses (Machado et al., 2015). The same tendency existed in the case of the post-fire losses of nitrate (NO_3-N), total nitrogen (TN), dissolved inorganic phosphorus (PO_4-P) and total phosphorus (TP) in these same two eucalypt plantations (Ferreira et al., 2016a; 2016b). Overall, plot-scale overland flow volumes and sediment losses in recently burnt eucalypt plantations in the study region can be explained well by rainfall amounts and/or intensities (Prats et al., 2012; Prats et al., 2014; Malvar et al., 2015).

Temporal patterns of DOC, POC and DIC concentrations

The temporal patterns in average POC, DOC and DIC concentrations at the two study sites (Figure 23) agreed well with the seasonal variations in the fractions' average exports. First, the highest concentrations of all three fractions occurred during autumn 2010, i.e. during the first months after the wildfire. Secondly, POC and DOC concentrations further revealed various minor peaks during winter 2010/11 and/or spring 2011, whilst DIC concentrations did not. Finally, DOC concentrations also showed several minor peaks towards the end of summer 2011, whilst POC and DIC concentrations did not. The temporal patterns in DOC and DIC concentrations were more complex than those in cation concentrations reported by Machado et al. (2015) for two eucalypt plantations in the same burnt area, with especially Na^+, K^+ and Mg^{2+} concentrations in micro-plot scale runoff decreasing sharply over the first two or three read-outs and basically remaining unaltered afterwards. The DOC patterns, however, bore considerable resemblance to the variations in dissolved inorganic phosphorus (PO_4-P) concentrations observed by Ferreira et al. (2016a) for the same plots as Machado et al. (2015).

The exact timing of the maximum concentration during autumn 2010 was markedly different for POC than for DOC and DIC fraction. DIC and DOC concentrations were highest on the first or second read-out with significant rainfall (08/09/2010 and 04/10/2010 with 21 and 56 mm, respectively). In contrast, POC concentrations not only peaked later but also on different dates at the two sites, i.e. on 03/11/2010 at the SE site (EB-S1) as opposed to 17/11/2010 at the NW site (EB-S2). This time lag could be explained by an earlier exhaustion of the easily erodible ashes at the SE than NW site, as cumulative POC and DOC exports by 03/11/201 amounted to 48 % of the initial ash TOC stock at EB-S1 as opposed to 14 % at EB-S2.

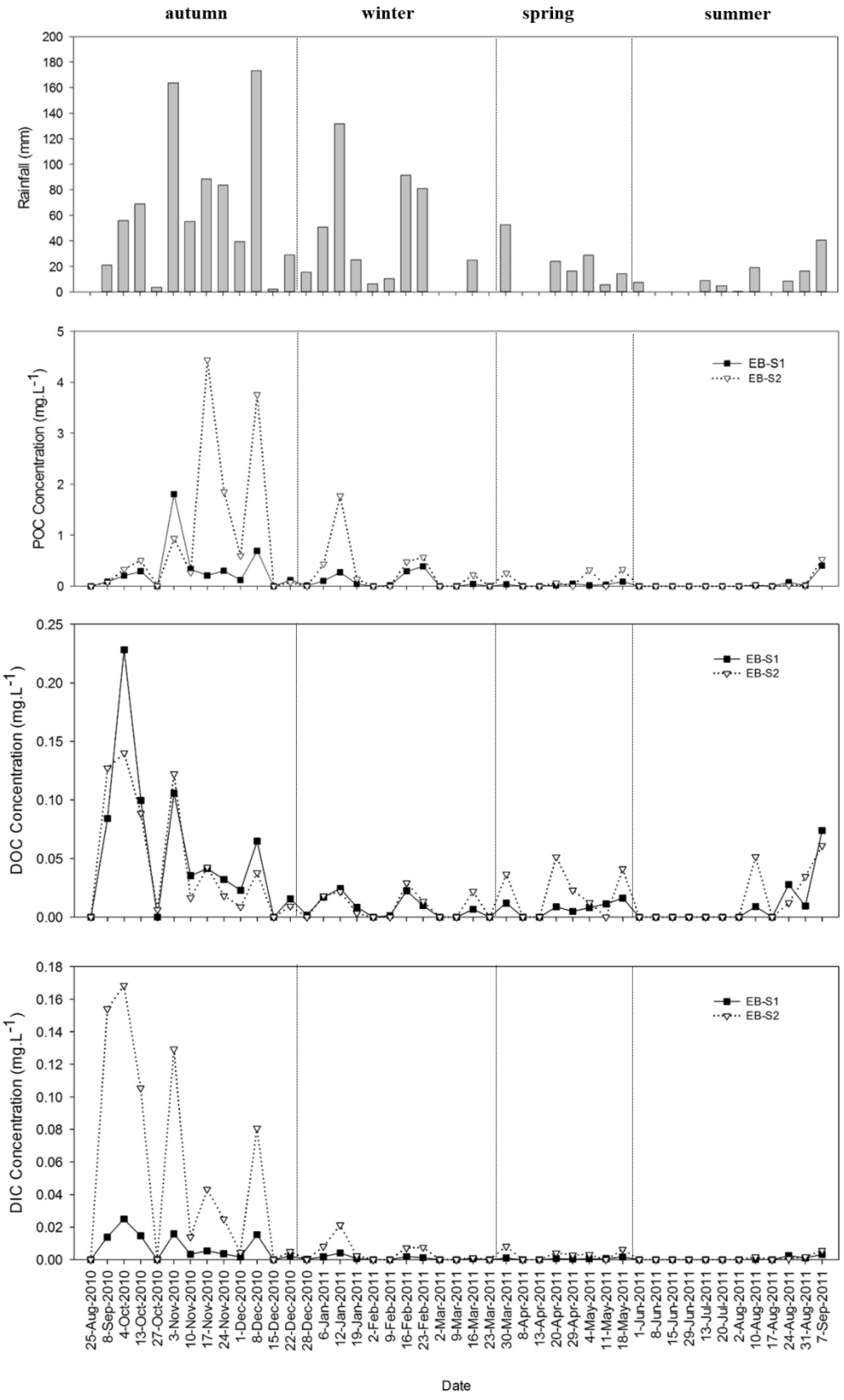

Figure 23 – Rainfall amount and dissolved organic carbon (DOC), particulate organic carbon (POC) and dissolved inorganic carbon (DIC) concentrations in overland flow at the two burnt eucalypt sites during the first year after the fire.

In line with the between-site differences in average total exports, maximum runoff concentrations of POC and DIC were, on average, clearly higher at the NW than SE slope. These maximum POC and DIC values amounted to 4.4 and 0.2 $g.L^{-1}$ at EB-S2, and to 1.8 and 0.03 $g.L^{-1}$ at EB-S1, respectively. In contrast, the maximum average DOC concentration was higher at the SE than NW slope (0.2 vs. 0.1 $g.L^{-1}$). These maximum DOC concentrations at both sites, however, were much higher (16-17 times) than the maximum DOC value of 8.4 $mg.L^{-1}$ found by Wagner et al. (2015) at the their most fire-affected catchment outlet. This discrepancy could be related to measurement scale, as argued by Keizer et al. (2015) with respect to the organic matter content of post-fire sediment export at the plot vs. catchment scale. On the other hand, Wagner et al. (2015) hypothesized that little input of sediments from the burnt area into the stream had occurred.

Conclusions

The present work evaluated post-fire soil organic carbon losses by overland flow at the micro-plot scale in recently burnt Mediterranean area, addressing a topic that has seldom been investigated. The main findings of the present work were that:

- The amount of deposited ashes was higher at the NW slope than at the SE slope, which suggested differences in the pre-fire loads. However, the total organic carbon content did not differ at the two sites.
- The total organic carbon content was similar at the both sites. However, the soil organic carbon stock was larger at the NW site, which could be explained by the lower stone content observed in topsoil of this slope.
- Total sediment losses were three times higher at the NW slope than at the SE slope, suggesting the existence of protective cover layer that considerably reduced overland flow generation and the associated sediment losses.
- The total organic carbon exports were also greater at the NW slope than at the SE slope and, largely determined by the particulate organic carbon fraction.
- The fractions temporal patterns (particulate organic carbon and dissolved organic carbon) generally decreased with time-since-fire and, particularly intense in the first months after the fire, which could be explained by the first post-fire intense rainfall events.

In addition, more studies are recommended to address broader spatial scales as well as wider time scales to fully understand post-fire organic carbon dynamics in the recently burnt areas.

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Chapter 3

Other contributions

Martins, M.A., Machado, A.I., Serpa, D., Prats, S.A., Faria, S.R., Varela, M.E., González-Pelayo, Ó., Keizer, J.J., 2013. Runoff and inter-rill erosion in a Maritime Pine and a Eucalypt plantation following wildfire and terracing in north-central Portugal. Journal of Hydrology and Hydromechanics, 61, 261-268.

Abstract

The purpose of this study was to assess how terracing affected overland flow and associated sediment losses, at the micro-plot scale (0.25 m^2), in recently burnt stands of the two principal forest types in north-central Portugal, i.e. mono-specific stands of maritime pine and eucalypt. Terracing is an increasingly common practice of slope engineering in the study region but its impacts on runoff and erosion are poorly studied. Non-terraced plots at the eucalypt and the pine site revealed similar median runoff coefficients (rc: 20–30%) as well as comparable median sediment losses (15–25 g m^{-2}) during the first seven months following wildfire. During the ensuing, slightly wetter 18-month period, however, non-terraced plots at the Pine site lost noticeably more sediments (in median, 90 vs. 18 g m^{-2}), in spite the runoff response had remained basically the same (median rc: 33 vs. 28%). By contrast, terraced plots at the same Pine site lost hugely more sediments (in median, 1,200 g m^{-2}) during this 18-month period. Terraced plots at the eucalypt site even lost three times more sediments (in median, 3,600 g m^{-2}). Ground cover and resistance to shear stress seemed to be key factors in the observed/inferred impacts of terracing.

Keizer, J.J., Martins, M.A., Prats, S.A., Faria, S.R., González-Pelayo, O., Machado, A.I., Rial-Rivas, M.E., Santos, L.F., Serpa, D., Varela, M.E., 2015. Within-in flume sediment deposition in a forested catchment following wildfire and post-fire bench terracing, north-central Portugal. Quadernos De Investigación Geográfica. 41, 1, 149-164.

Abstract

Wildfires increase plot-scale soil erosion rates across the world. However, very few studies have monitored post-fire erosion losses at the catchment scale, especially in the Mediterranean region. The FIRECNUTS project was originally set out to address this research gap by selecting a recently burnt catchment and instrumenting its outlet with two flumes with maximum discharge capacities of 120 and 1700 l s^{-1}. Six months after the fire, however, this experimental catchment was bench terraced by bulldozer. Furthermore, the smallest flume suffered from regular deposition of sediments from the onset of its construction. This study was therefore an attempt to explore whether quantifying this deposition of sediments could provide further insight into the sediment yield of a recently burnt and terraced catchment. To this end, the deposited sediments were removed and weighted on a total of 101 occasions during a three year period. The results revealed that: (i) the amounts of sediment deposited over the three hydrological years corresponded to relatively small erosion rates, varying between 0.030 and 0.046 Mg ha^{-1} yr^{-1}; (ii) while bench terracing is generally viewed as a soil and water conservation technique, it did not produce a clear decrease in sediment deposition, as deposition per mm of streamflow was the same before and after terracing (0.8 kg mm^{-1}); (iii) daily sediment deposition could be explained reasonably well by daily rainfall volume and maximum daily streamflow volume.

Chapter 4

General conclusions and future perspectives

This research provides an understanding into the short-term effects (direct and indirect) of wildfires on the quantity and quality of soil organic matter (SOM) and soil organic carbon (SOC) in burnt forest areas in north-central of Portugal.

Quantification of post-fire of soil organic carbon (SOC) content and evaluation of fire-induced changes on the molecular structure of soil organic matter (SOM) have become increasingly important to improve the understanding of the global C cycle. Furthermore, overall wildfires effects on SOM, is required for a more accurate assessment of the risks of post-fire soil fertility and its possible impacts on forest recovery. This topic has seldom been addressed, which is an important research gap since wildfire frequency, severity and the size of burnt areas are not expected to decline markedly in foreseeable future. Therefore, this work aimed to cover this gap by providing an understanding into the short-term effects (direct and indirect) of wildfires on the quantity and quality of SOM and SOC in burnt forest areas in north-central Portugal.

The main findings of this research were that:

- Since SOM is an heterogeneous mixture of molecules in variable proportions and evolution status, therefore a combination of multi-analytical approach, such as lipid-biomarkers analysis by GC/MS, SOM characterization by Py-GC/MS and ^{13}C NMR spectroscopy, is an appropriate method to provide an integrated understanding of SOM composition, dynamics, and alterations caused by forest fires (Table 15);

- Analytical pyrolysis allows the detection of SOM differences between burnt and unburnt topsoil from north-central Portugal. Article I (chapter 2.1) was the first exploratory approach of these analytical techniques to study wildfire-induced changes on SOM composition for distinct Portuguese forest types (pine and eucalypt) one year after the fire, focussing especially on the role of possible differences in fire severity. This study showed some differences between burnt and unburnt topsoil due fire-induced SOM modifications, such as (i) reduction in the more thermally-labile molecular structures and (ii) an accumulation of low-molecular weight homologues of certain chemical series, suggesting the

occurrence of thermal breakdown and cracking of long-chain SOM components exerted by fire. However differences between the two forest types were not clear.

- The biomarkers identified in this study of the soil lipid fraction (article II – chapter 2.2) indicated that the wildfire modified SOM by (i) thermal breakdown of the alkyl-chains, increasing the ratios of short-to-long *n*-fatty acids and *n*-alkanes; (ii) increasing and altering the characteristic CPIs; (iii) increasing the relative abundance of aromatic compounds, most probably through the input of charred biomass; (iv) decreasing the relative abundances of specific biomarkers of vegetation, in particular, *Eucalyptus globulus,* such as the terpenoids epiglobulol, ledol and globulol; (v) introducing compounds that are thermal degradation products from steroids, such as stigmastan-3,5-diene (derived from β-sitosterol); (vi) increasing the relative abundance of lignin compounds, indicating the greater thermal stability of lignin moieties compared with other SOM chemical structures; (vii) introducing levoglucosan, indicating thermal alteration of biomass; and (viii) introducing heteroaromatic N-compounds, pointing towards a thermal transformation of SOM.

  The biogeochemical markers allowed studying the evolution of the wildfires effects on soils. The findings obtained in time-since-fire chrono-sequence of soil properties are relevant from a management point-of-view i.e. knowledge about SOM variables indicating a recovery of soil after a wildfire may be useful to optimize the efficacy of restoration actions. A better understanding of the relationship between vegetation and SOM molecular composition will allow predicting the stability of soil C pools with different mean resident times.

- In the article III (chapter 2.3) the application of analytical pyrolysis allowed the assessment of the remarkable SOM differences between burnt and unburnt topsoil and also the eroded sediment exports by overland flow during two years after the fire in burnt eucalypt plantations (Table 15). These effects involved: (i) an enrichment of aromatic compounds and especially, PAHs derived from charcoal

and partially charred OM, as well as an additional enrichment of the eroded sediments, which should be duly considered due to its abundance of persistent organic pollutants; (ii) increases in heterocyclic N together with an increase in the short-to-long ratio of *n*-alkanes, and reduction in average chain length, all of which indicated thermal transformation of fire affected SOM; (iii) a decrease of biomarkers of *Eucalyptus globulus,* as well as of other terpenoids used as specific biomarkers of vegetation, which is consistent with noticeable fire-induced changes in vegetation cover; and (iv) partial recovery of the topsoil SOM quality.

The ^{13}C NMR spectroscopy was also used for complementing the results of analytical pyrolysis, which showed an increase in aromaticity and also revealed a slow soil recovery in the burnt topsoils during the first two years after the fire.

- In the article IV (chapter 2.4) evaluated post-fire soil organic carbon losses by overland flow at the micro-plot scale in recently burnt Mediterranean area, addressing a topic that has seldom been investigated. The main findings of the present work were that: (i) the amount of deposited ashes was higher at the NW slope than at the SE slope, which suggested differences in the pre-fire loads. However, the total organic carbon content did not differ at the two sites; (ii) the total organic carbon content was similar at the both sites. However, the soil organic carbon stock was larger at the NW site, which could be explained by the lower stone content observed in topsoil of this slope; (iii) total sediment losses were three times higher at the NW slope than at the SE slope, suggesting the existence of protective cover layer that considerably reduced overland flow generation and the associated sediment losses (iv) the total organic carbon export were also greater at the NW slope than at the SE slope and, largely determined by the particulate organic carbon fraction; (v) the fractions temporal patterns (particulate organic carbon and dissolved organic carbon) generally decreased with time-since-fire and, particularly intense in the first months after the fire, which could be explained by the first post-fire intense rainfall events.

Estimated losses of total organic carbon calculated in relation to the stock of soil organic carbon ranged between 2-4%. In spite of apparently modest fire effect on

organic carbon losses, post-fire management efforts to minimize the loss of soil fertility are recommended.

The data collected within this study, has the potential to help understanding additional research gaps, especially regarding microbiological activity, post-fire runoff and erosive response. In addition, some soil water repellency relations can be predicted using hydrophobic organic compounds and their vegetation source. The integrated fieldwork installation in the present study area can be used to analyse different soil depths, or larger scales than micro plot and the analysis be upscalled to hillslopes or to the entire catchment. This would allow understanding the transport and distribution of organic carbon in different environmental compartments.

Understanding the functions and dynamics of organic matter is essential for ecosystem management and sustainability. Furthermore, findings obtained in time-since-fire chrono-sequence of soil properties are relevant from a management point-of-view i.e. knowledge about SOM variables, which indicate a recovery of soil after a wildfire could be useful to optimize restoration actions.

Table 15 - Synthesis of fire effects on the soil organic matter.

	Burnt topsoil (compared with unburnt topsoil)	***Eroded Sediments (compared with burnt topsoil)***
Elemental analysis	*pH* • immediately after the fire: increase of pH value • two years after the fire: remained slightly higher *Total organic carbon (TOC)* • immediately after the fire: decrease of the TOC content • two years after the fire: remained lower *Total nitrogen (TN)* • immediately after the fire: increase of the TN content • two years after the fire: remained lower *Total lipids extract (TLE)* • immediately after the fire: higher decrease of the TLE • two years after the fire: remained lower	*pH* • immediately after the fire : increase of pH value • two years after the fire : remained slightly higher *Total organic carbon (TOC)* • immediately after the fire : increase of the TOC content • two years after the fire : remained highest *Total nitrogen (TN)* • immediately after the fire : increase of the TN content • two years after the fire : remained highest *Total lipids extract (TLE)* • *n.d.* *Cumulative sediments losses* • Two years after the fire : 1.3 Mg ha^{-1} y^{-1} • Unburnt topsoil: 0.046 Mg ha^{-1} y^{-1}
Lipid-biomarkers	*Increase of:* • the ratios of short-to-long *n*-fatty acids and *n*-alkanes • relative abundance of aromatic compounds (input of charred biomass) • the relative abundance of lignin compounds (greater thermal stability of lignin moieties compared with other SOM chemical structures)	*n.d.*

	Decrease of: • relative abundances of specific biomarkers of *Eucalyptus globulus* (such as the terpenoids: epiglobulol, ledol and globulol) *Other observations:* • thermal breakdown of the alkyl-chains • Appearance of thermal degradation products from steroids (such as stigmastan-3,5-diene (derived from β-sitosterol) • Appearance of levoglucosan and heteroaromatic N-compounds (pointing thermal alteration of SOM) • limited post-fire recovery of soil over two years	
Py-GC/MS	*Increase of:* • aromatic compounds (especially PAHs, persistent organic pollutants; derived from charcoal and partially charred organic matter • heterocyclic N compounds and an increase of the short-to-long ratio of n-alkanes (thermal transformation of the fire affected SOM) *Decrease of:* • in the Average Chain Length (ACL) • relative abundances of specific biomarkers of *Eucalyptus globulus* *Other observations:* • Partial recovery of the topsoil SOM quality	
^{13}C NMR	*Increase of:* • relative abundance of aromatic compounds *Other observations:* • weak enrichment in the relative abundance of carbohydrates • limited post-fire recovery of soil over two years	*n.d*

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